CRYSTALLOGRAPHIC CALCULATIONS ON THE SILLIAC ELECTRONIC DIGITAL COMPUTER

I. FOURIER SYNTHESES

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Summary

A method is presented whereby SILLIAC, a high-speed electronic digital computer, sums 2-dimensional Fourier syntheses for crystallographic electron-density projections of any symmetry. The trigonometrical form of a particular summation and the area over which the projection is to be calculated are determined by programme-parameters introduced with the data.

I. INTRODUCTION

The use of punched card and punched tape methods for crystallographic calculations is now fairly common (e.g. Shaffer, Schomaker, and Pauling 1946; Bennett and Kendrew 1952; Ordway 1952; Ahmed and Cruickshank 1953; Lavine and Rollett 1956; Sparks *et al.* 1956). The particular developments which are reported here are an extension of the method adopted by Bennett and Kendrew for the summation of 2-dimensional Patterson functions on EDSAC. The present programme has been developed for SILLIAC (the Sydney University version of the University of Illinois Automatic Computer). By means of it, Fourier syntheses for electron-density projections of any symmetry may be completed in about 5 min of computer time for 200 structure factors summed over a quarter of a unit-cell at $\frac{1}{64}$ths of the cell edges. SILLIAC's present limitation lies in its comparatively short "memory". The extension of the Fourier programme to include the calculation of 3-dimensional functions has accordingly been deferred, pending the addition to SILLIAC of a magnetic tape backing store.

Like EDSAC, SILLIAC is a high-speed electronic digital computer, which can perform the normal arithmetical operations by means of single-address orders. These orders are stored in pairs in any of the 1024 memory locations, each of which holds a number or order-pair of up to 40 binary digits (or "bits"). The operations of addition-subtraction, division, multiplication, and multiplication by $2^{\pm n}$ take, respectively, 75, 800, 700, and $16n$ μsec. (For comparison, these times are approximately the same as those for SWAC (Sparks *et al.* 1956), while the corresponding times reported for EDSAC operations were larger by factors of about 10.)

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SILLIAC also recognizes orders which cause the machine to choose one or other of two sequences of operations depending on the sign of a number in the accumulator; these orders take 18 or 55 μsec, depending on the nature of the recognized sign. In addition, since orders are stored in the machine in the same way as numbers, they, too, may be modified by machine operations. Input and output of orders, data, and results occur by means of 5-hole characters punched on a tape, which may be converted to printed form by means of a teleprinter. The rate of input is 200 characters per sec, that of output is at present 60 characters per sec.

The programme for 2-dimensional Fourier summations sums the series

$$\rho(x,y)=\sum_{h=0}^{h_{\max.}}\sum_{k=0}^{k_{\max.}}F_{hk}\begin{Bmatrix}\cos\\ or\\ \sin\end{Bmatrix}2\pi ky/b\cdot\begin{Bmatrix}\cos\\ or\\ \sin\end{Bmatrix}2\pi hx/a,$$

where $\rho(x,y)$ is the projected electron-density at (x,y), and F_{hk} is the structure factor for the (hk) reflection. The function is computed at intervals of $\frac{1}{64}$ of each cell-edge. In the y-direction the summation is from 0 to a specified $y_{\max.}$, in the x-direction from 0 to a specified $x_{\max.}$ or from $-x_{\max.}$ to $+x_{\max.}$. The maximum value of h is 29; and the parameter $h_{\max.}$ must be specified for each computation. There is no limitation on the value of the index k.

Since h and k can each be even or odd, only four possible combinations can arise, viz. both odd; h odd, k even; h even, k odd; both even. SILLIAC tests the h and k indices of each F_{hk} and chooses the correct one out of the possible four combinations of trigonometrical factors for the F_{hk} being summed, according to parameters which are supplied with each set of data.

II. Input of Data

The data are arranged on the tape as follows: The F_{hk}'s having a common value of h are grouped together in order of increasing k. Each F_{hk} is followed by its sign. The last non-zero F_{hk} of each group is followed by the symbol L, and the last non-zero F_{hk} of the group whose h is equal to $h_{\max.}$ is followed by the symbol F. One or more unobserved F's are indicated by the appropriate integer followed by the character N.

A second group of F_{hk} for summation with different trigonometrical functions selected according to different conditions from those for the first group, may be stored in the same way.

The numerical data are followed by the parameters:

$$h_{\max.},\ x_{\max.},\ y_{\max.},$$

and by four order-pairs representing the instructions for trigonometrical product formation for each of the four possible combinations of even- or odd-ness of h and k. A second set of four order-pairs may be inserted if necessary, for the second set of F's.

The total number of F's which may be summed is 550. Zero-values must be included in the list of data, though some economy is achieved by storing a

sequence of zero-F's in one storage location, as described above. The F's may have any positive or negative integral value; however, the scale must be such that $|\rho(x,y)|$ at no point exceeds 9999. Where a F_{hk} is to enter the summation in a negative product (e.g. $-F \sin 2\pi ky/b \,.\, \sin 2\pi hx/a$), the actual value of F must be multiplied by -1 before being submitted to the machine.

III. Outline of Computation

As in Bennett and Kendrew's case, the computation follows the commonly-used Lipson-Beevers' method. In Stage I two sets of $(h_{\max.}+1)$ terms are calculated—that is, $A(h,y)$ and $B(h,y)$—*both of which* have the form

$$\sum_{k=0}^{k_{\max.}} F_{hk} \begin{Bmatrix} \sin \\ or \\ \cos \end{Bmatrix} 2\pi ky/b.$$

Each product is included in one or other of these two sets; its allocation determines its fate in Stage II, in which

$$C = \sum_{h=0}^{h_{\max.}} A(h,y) \cos 2\pi hx/a,$$

and

$$D = \sum_{h=0}^{h_{\max.}} B(h,y) \sin 2\pi hx/a$$

are computed for the specified number of points in the x-direction. Finally,

$$\rho(x,y) = C + D,$$

and

$$\rho(-x,y) = C - D$$

are punched out for all the values of x/a at a constant y/b. The programme then changes y/b to a new value and the whole cycle is repeated. For projections which are symmetrical about the y-axis, the calculation and punching of $\rho(-x,y)$ may be suppressed.

The trigonometrical functions are found by means of a sub-routine which finds, and gives an appropriate sign to, the correct entry in a table of 17 values of $\sin 2\pi n/64$, $n=0$ to 16. This table is calculated and stored by the machine during the input of the programme.

The values of h and k for any F_{hk} are found by the machine by means of storage locations used as appropriate counters. At the beginning of each cycle (i.e. whenever y/b has been changed to a new value) the numbers in the h- and k-counters are reduced to zero. As each normal F_{hk} enters the summation k is merely increased by unity. When an integer originally followed by N occurs (indicating a sequence of zero-F's), no product calculation takes place but k is correspondingly increased. Whenever a F_{hk} on the data-tape is followed by L, the h-counter for the following F_{hk} will be increased by unity and the k-counter reduced to zero.

IV. Calculation Time

The time used by SILLIAC for the actual calculation is given roughly by the expression

$$(y_{\max.}+1)[8\cdot5(\text{No. of } F\text{-terms})+3\cdot5(h_{\max.}+1)(x_{\max.}+1)] \text{ msec.}$$

For a Fourier projection of 150 terms, with $0\leqslant h\leqslant 11$, summed for $-32/64\leqslant x/a\leqslant 32/64$ and $0\leqslant y/b\leqslant 16/64$, the above expression gives a calculation time of 45 sec. The calculations overlap to a small extent with the much slower punching of the results, so that the total time (min) is occupied by

Input of programme and data	1
Computation and output	4·5

To these times (min) must be added

Data-tape punching	10
Data-tape checking	4
Print-out of results-tape	9
Total	28·5

When successive Fourier projections differ only in a small number of the signs of the coefficients, the preparation of all the data tapes after the first can, of course, be achieved much more rapidly by the use of the available tape-editing equipment.

V. Acknowledgments

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RAPID REACTION BETWEEN UNIFUNCTIONAL MOLECULES AND MOLECULES UNIFUNCTIONAL AT ONE POSITION AND n-FUNCTIONAL AT ANOTHER

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Summary

Rate equations for the reaction between A and B, where B is unifunctional, and A is unifunctional at one and n-functional at the other of two independent positions, lead to expressions for the final relative concentrations of the reactants and products in terms of the initial concentrations of A and B, the ratio of the velocity constants at the two positions on A, and the functionality n. These results are applied to the reaction of tryptophane with p-diazobenzenesulphonic acid, in which it appears that the indole group reacts somewhat faster than the amino group.

I. Introduction

In this paper the treatment presented by Higgins and Williams (1953—referred to below as " the 1953 paper ") to describe the kinetics of rapid reactions involving two pairs of consecutive second-order steps is partially generalized to describe a more complex type of reaction. The modifications, although mathematically simple, are not evident from the 1953 treatment and extend the range of application of the method in the study of the relationship between molecular structure and reactivity.

The reaction is assumed to take place irreversibly in the following stages :

$$(1) \quad A + B \xrightarrow{k_1} AB,$$

$$(2) \quad AB + nB \xrightarrow{k_2} B_nAB,$$

$$(3) \quad A + nB \xrightarrow{k_3} B_nA,$$

$$(4) \quad B_nA + B \xrightarrow{k_4} B_nAB,$$

where the reactant A is capable of unifunctional behaviour at one of two reactive positions and n-functional behaviour at the other and B is exclusively unifunctional, and where k_1, k_2, k_3, and k_4 are the velocity constants of the various stages. A can react initially with B at either of the two positions to produce AB or B_nA, both of which are capable of reacting with B at the remaining position to produce B_nAB.

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For the purposes of this treatment it will be considered that stages (2) and (3) are each controlled by the first of a series of n second-order substages, as shown:

		Stage (2)	Stage (3)
Substage:	(1)	$AB+B \overset{k_2}{\rightarrow} BAB$,	$A+B \overset{k_3}{\rightarrow} BA$,
	(2)	$BAB+B \rightarrow B_2AB$,	$BA+B \rightarrow B_2A$,
		. . . . .	. . . . .
	(n)	$B_{n-1}AB+B \rightarrow B_nAB$,	$B_{n-1}A+B \rightarrow B_nA$,

The velocity constants of substages (2) to (n) must each be sufficiently greater than that of the preceding substage for the concentrations of the intermediates $B_{<n}AB$ and $B_{<n}A$ not to enter into the argument. (As this may rarely have much practical significance for $n>2$, special attention is paid to the case when $n=2$, although most of the equations are given in the more general form.) The assumption of a sequence of increasing velocity constants for the various substages is equivalent to regarding the second position on molecule A as *exclusively* n-functional.*

II. Theory

(a) *General*

Let c with appropriate subscript denote the molar concentrations at time t and x_1, x_2, x_3, x_4 denote the fall in the concentrations of A, AB, A, and B_nA respectively in time t due to the individual reactions 1, 2, 3, and 4. The initial concentrations of A and B are a and b respectively and of AB, B_nA and B_nAB are zero. Then

$$a-(x_1+x_3) = c_A, \quad \ldots\ldots\ldots\ldots \quad (1)$$
$$b-(x_1+x_4)-n(x_2+x_3) = c_B, \quad \ldots\ldots\ldots\ldots \quad (2)$$
$$x_1-x_2 = c_{AB}, \quad \ldots\ldots\ldots\ldots \quad (3)$$
$$x_3-x_4 = c_{B_nA}, \quad \ldots\ldots\ldots\ldots \quad (4)$$
$$x_2+x_4 = c_{B_nAB}. \quad \ldots\ldots\ldots\ldots \quad (5)$$

Rate equations may be set up as follows:

$$dx_1/dt = k_1(a-x_1-x_3)(b-x_1-x_4-nx_2-nx_3), \quad \ldots\ldots \quad (6)$$
$$dx_2/dt = k_2(x_1-x_2)(b-x_1-x_4-nx_2-nx_3), \quad \ldots\ldots\ldots \quad (7)$$
$$dx_3/dt = k_3(a-x_1-x_3)(b-x_1-x_4-nx_2-nx_3), \quad \ldots\ldots \quad (8)$$

and

$$dx_4/dt = k_4(a-x_1-x_3)(b-x_1-x_4-nx_2-nx_3). \quad \ldots\ldots \quad (9)$$

In view of the restriction imposed in the last two paragraphs of Section I, the c_B terms in equations (7) and (8) are of the first degree only, so that after eliminating t, the equations reduce to the same form as in the 1953 paper. These no longer involve n, and the general solutions are given by equations (15) and (16) of the 1953 paper.

* See footnote to Section III on p. 108.

(*b*) *Final State*

(i) When $a<b/(n+1)$: Here $x_1+x_3=a$, $x_1=x_2$, and $x_3=x_4$, so that final concentrations C, with appropriate subscript, are given, from equations (**1**) to (**5**), by

$$C_A=C_{AB}=C_{B_nA}=0\,; \quad C_B=b-(n+1)a\,; \quad C_{B_nAB}=a. \qquad \ldots\ldots \quad (10)$$

(ii) When $a>b/(n+1)$: Here

$$x_1+x_4+n(x_2+x_3)=b, \qquad \ldots\ldots\ldots\ldots \quad (11)$$

and $C_B=0$.

If the two positions on molecule A are virtually independent we can take $k_1=k_4$ and $k_2=k_3$, and putting $K=k_2:k_1$ we get equations (**19**) and (**20**) of the 1953 paper. Upon applying our new final condition (eqn. (**11**) present paper) for $a>b/(n+1)$, we get eventually

$$\frac{b}{a}=n+1-n\left(\frac{C_A}{a}\right)^{K/(1+K)}-\left(\frac{C_A}{a}\right)^{1/(1+K)}; \qquad \ldots\ldots\ldots \quad (12)$$

(when $n=1$, this reduces to eqn. (**24**) of the 1953 paper). To relate C_A, C_{AB}, C_{B_nA}, and C_{B_nAB} we can derive equations which are the same as equations (**25**) to (**28**) of the 1953 paper, except that C_{B_nA} and C_{B_nAB} now replace C_{BA} and C_{BAB} respectively. Since these are not dependent on the final state condition no further generalization is required to account for the n-functionality.

TABLE 1

EXPRESSIONS FOR FINAL CONCENTRATIONS WHEN THE RATIO OF THE VELOCITY CONSTANTS IS VERY LARGE, BUT NOT INFINITE

Final Concentration	$k_1 \gg k_2$		$k_2 \gg k_1$	
	$a>b$	$b>a>b/(n+1)$	$a>b/n$	$b/n>a>b/(n+1)$
C_A	$a-b$	0	$(na-b)/n$	0
C_B	0	0	0	0
C_{AB}	b	$[(n+1)a-b]/n$	0	0
C_{B_nA}	0	0	b/n	$(n+1)a-b$
C_{B_nAB}	0	$(b-a)/n$	0	$b-na$

When $K=0$ or ∞ the problem of determining the final concentrations becomes trivial. However with K very large or very small, but finite, with b constant and $a>b/(n+1)$, the relationship between the final concentration of each of the various reactants and products and the initial concentration of A can be represented by straight lines with a change in slope at critical values of a. Consideration of the physical mechanism along the same lines as in the 1953 paper, but necessarily in more detail, leads to the boundary conditions and to the expressions for the final concentrations of reactants and products given in Table 1.

Since n may be expected to be a small integer and will rarely exceed 2, the form taken by equation (**12**) when $n=2$,

$$\frac{b}{a}=3-2\left(\frac{C_A}{a}\right)^{K/(1+K)}-\left(\frac{C_A}{a}\right)^{1/(1+K)} \quad \ldots\ldots\ldots \textbf{(13)}$$

is of special practical interest. Figures 1 and 2 show the dependence of the final relative concentrations of reactants and products on the ratio of the initial concentrations of A and B for various ratios of the velocity constants. It is convenient, when dealing with systems in which the initial concentration of the

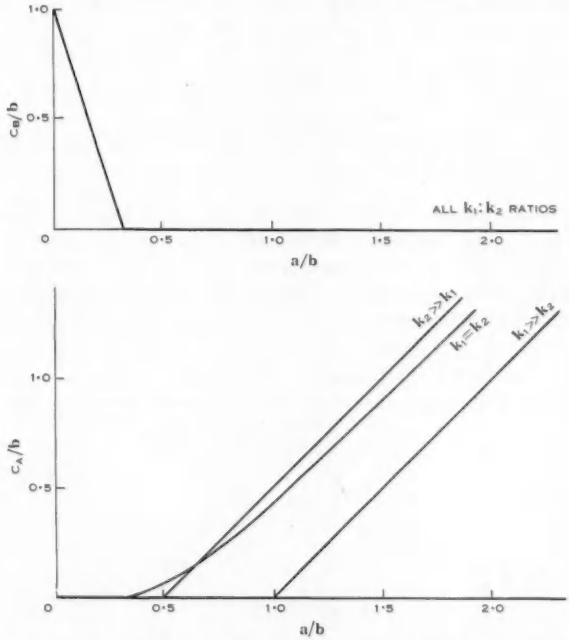

Fig. 1.—Final relative concentrations of reactants A (below) and B (above) for $n=2$.

$(n+1)$-functional molecule is varied and that of the unifunctional molecule is constant, to express the final concentrations as a function of the initial molar ratio a/b. For this purpose C/a has in each case been replaced by C/b, which, with b constant, is proportional to C, and the figures have been plotted on this basis.

For AB and B_nA the curves for intermediate finite values of K fall within the rectilinear limits defined by the two curves for the extreme finite K values. For A and B_nAB, however, some combinations of K and a/b will give values of C_A/b and C_{B_nAB}/b falling outside the corresponding rectilinear limits. It

can be shown empirically that the curves for $K=1$ represent the boundaries of such excursions. By equating the expression for C_A when $K=1$, from equation (12), to the appropriate value when $k_2 \gg k_1$ (from Table 1) it may be shown that the values for C_A coincide when

$$\frac{a}{b}=\frac{n}{n^2-1}.$$

Similarly, from equation (12), equation (28) of the 1953 paper, and Table 1, it may be shown that the C_{B_nAB} *v.* a/b curves for $K=1$ and $k_1 \gg k_2$ meet when

$$\frac{a}{b}=\frac{n}{n+1} \text{ or } \frac{1}{n+1}.$$

In Figures 1 and 2 (for $n=2$), this coincidence occurs at $a/b=2/3$ and at $a/b=1/3$ and $2/3$ respectively.

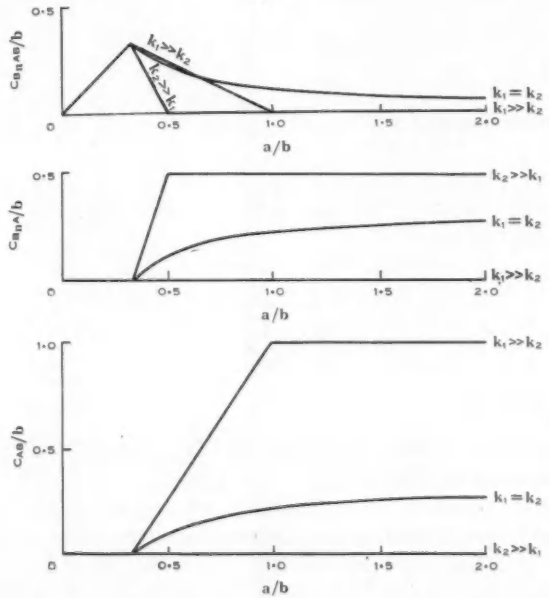

Fig. 2.—Final relative concentrations of products AB (below), B_2A (middle), and B_2AB (above) for $n=2$.

(*c*) *Constant Initial Molar Ratio of Reactants*

The initial concentrations of the reactants are usually a matter of the experimenter's choice, and so for determining relative reactivities by competition for molecules of B at the two reactive sites on A it is sufficient in principle to keep a/b constant. The molar ratio should be so chosen that the component to be determined will have a reasonably large relative concentration as the system approaches equilibrium and so that errors in measuring the concentration

will give rise to the minimum errors in K. These conditions are usually fulfilled when the initial molar concentrations of the two reactants are about the same.

When a/b is constant the following expressions may be derived from equation (**12**) in a similar way to that used in Section II (d) of the 1953 paper:

$$K=\log\left(1-\frac{y}{n}-\frac{b}{an}+\frac{1}{n}\right)/\log y, \quad \ldots\ldots\ldots\ldots \quad (14)$$

$$\frac{C_A}{a}=y\left(1-\frac{y}{n}-\frac{b}{an}+\frac{1}{n}\right), \quad \ldots\ldots\ldots\ldots\ldots \quad (15)$$

$$\left.\begin{aligned}\frac{C_{AB}}{a}&=1-\frac{y}{n}-\frac{b}{an}+\frac{1}{n}-\frac{C_A}{a}\\&=(1-y)\left(1-\frac{y}{n}-\frac{b}{an}+\frac{1}{n}\right)\end{aligned}\right\}, \quad \ldots\ldots\ldots\ldots \quad (16)$$

$$\left.\begin{aligned}\frac{C_{B_nA}}{a}&=y-\frac{C_A}{a}\\&=y\left(\frac{y}{n}+\frac{b}{an}-\frac{1}{n}\right),\end{aligned}\right\}, \quad \ldots\ldots\ldots\ldots\ldots \quad (17)$$

$$\left.\begin{aligned}\frac{C_{B_nAB}}{a}&=\frac{C_A}{a}-y+\frac{y}{n}+\frac{b}{an}-\frac{1}{n}\\&=(1-y)\left(\frac{y}{n}+\frac{b}{an}-\frac{1}{n}\right)\end{aligned}\right\}, \quad \ldots\ldots\ldots\ldots \quad (18)$$

where $y=(C_A/a)^{1/(1+K)}$.

Differentiation of equation (**18**) with respect to y shows that C_{B_nAB}/a passes through a maximum at $b^2/4a^2n$, where $y=1-b/2a$.

When $a/b=1$, equations (**14**) to (**18**) reduce to simpler terms; the relationships between the final relative concentrations and the ratio of the velocity constants for $n=2$ and $n=3$ are shown in Figure 3. Since K increases continuously with y, the C_{B_nAB}/a *v.* K curves also pass through a maximum, in this case when C_{B_nAB}/a is $1/4n$, that is, 1/8 and 1/12 respectively.

(*d*) *Final Concentration and Functionality*

It is of some interest to consider the variation in final concentrations with the functionality of the second position on molecule A, even though, in practice, it would be difficult, in order to check the dependence, to find compounds with the same K value but differing n. The theoretical relationship is shown in Table 2 for the simple case when $K=1$ and $a/b=1$. These figures were calculated from the following expressions (which were obtained by appropriate insertions

in equation (12) and from the equations relating the various final relative concentrations :

$$C_A/a = n^2/(n+1)^2, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (19)$$

$$C_{AB}/a = C_{B_nA}/a = n/(n+1)^2, \quad \ldots\ldots\ldots\ldots \quad (20)$$

$$C_{B_nAB}/a = 1/(n+1)^2. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (21)$$

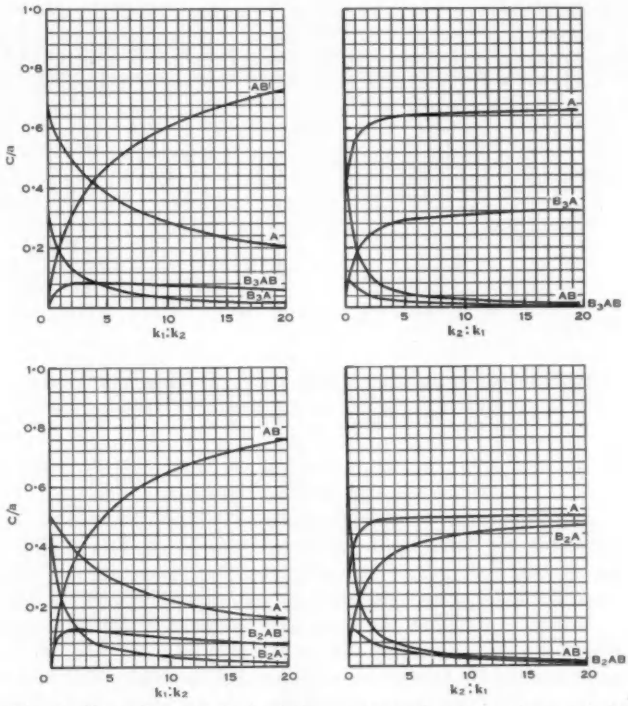

Fig. 3.—Dependence of final relative concentrations on ratio of velocity constants at independent reactive sites for the case of equal initial concentrations of reactants when $n=2$ (below) and $n=3$ (above).

It will be seen that $C_A : C_{AB}(=C_{B_nA}) = C_{AB} : C_{B_nAB} = n$, which results in coincidence when $n=1$.

When a/b is constant but $\neq 1$, we have if $K=1$

$$\frac{C_A}{a} = \frac{(n+1-b/a)^2}{(n+1)^2}, \quad \ldots\ldots\ldots\ldots\ldots \quad (22)$$

$$\frac{C_{AB}}{a} = \frac{C_{B_nA}}{a} = \frac{(b/a)(n+1-b/a)}{(n+1)^2}, \quad \ldots\ldots\ldots\ldots \quad (23)$$

and

$$C_{B_nAB}=\left(\frac{b/a}{n+1}\right)^2. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (24)$$

Here, $C_A : C_{AB}(=C_{B_nA})=C_{AB} : C_{B_nAB}=\frac{a}{b}\left(n+1-\frac{b}{a}\right)=\frac{a}{b}(n+1)-1$, which reduces to n when $a/b=1$, as in Table 2.

TABLE 2

THEORETICAL DEPENDENCE OF FINAL RELATIVE CONCENTRATION OF REACTANT A AND PRODUCTS ON FUNCTIONALITY OF SECOND POSITION ON MOLECULE A WHEN $K=1$, $a=b$

Functionality	Final Concentrations		
	A	AB, B_nA	B_nAB
1	0·2500	0·2500	0·2500
2	0·4444	0·2222	0·1111
3	0·5625	0·1875	0·0625
4	0·6400	0·1600	0·0400

The frequency with which $n+1$ appears in these equations is clearly related to its physical significance as the *overall* functionality of molecule A.

III. Application to the Reaction of Tryptophane with *p*-Diazobenzenesulphonic Acid

At this stage, applying the theory derived in Section II, we may introduce the problem which gave rise to it. This concerns the nature of the reaction between tryptophane (A) and *p*-diazobenzenesulphonic acid (B). The products to be considered can be designated as B_2A, AB, and B_2AB, in the symbols of the theoretical treatment (with $n=2$), B_2 and the final B referring respectively to the substituents at the amino and indole groups. No attempt has been made to isolate the reaction products, or to analyse them by other than spectrophotometric methods, and their structures are open to doubt. However, Busch, Patrascanu, and Weber (1934) identified the product of reaction between benzene diazonium chloride and glycine as *NN*-bis(benzenediazo)glycine, postulating the structure $(C_6H_5.N:N)_2NCH_2.COOH$. Eagle and Vickers (1936) reported that tryptophane reacted with diazonium salts at both the indole and the amino group. This is supported by our spectrophotometric evidence, the derivatives on these two groups giving rise to absorption bands at *c.* 270 and 363 mμ respectively.

Procedure.—Diazotization of sulphanilic acid by means of nitrous acid is followed by reaction of the diazotized sulphanilic acid with the amino acid. The reagents and procedure were the same as previously employed by Higgins and Fraser (1952), but without ethanol in the system ; the initial concentration of sulphanilic acid was kept constant at 2·31 mmoles/l. The amino acid concentra-

tion was varied over a wide range so as to produce a series of mixtures of the various azo-derivatives. Both the amino acid and the diazotized sulphanilic acid are present in the system before their interaction commences, this being sponsored by the addition of alkali (sodium carbonate), with mixing. Thus the reactant in excess at all stages was in fact determined by the total concentrations used. The absorption spectra or individual extinctions were measured $\frac{1}{2}$ to 1 hr after the addition of the alkali. Examination of the time-dependence of the extinctions showed that these reactions, although rapid enough for final state kinetics to be applied to measurements made after *c.* 10 min, are much slower than the virtually " instantaneous " coupling between diazonium compounds and phenolic or imidazole groups.

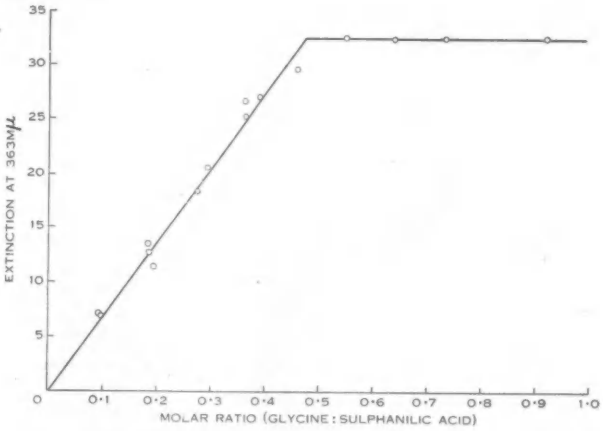

Fig. 4.—Dependence of extinction at 363 mμ, due to azo-compound, upon initial glycine concentration, showing conformity with Beer's law up to a molar ratio of nearly 1 : 2.

Glycine was shown to react with diazotized sulphanilic acid to yield a product with only one absorption band (maximum at 363 mμ), even at high glycine concentrations. Saturation extinction is attained at a molar ratio of glycine : sulphanilic acid of *c.* $\frac{1}{2}$ (Fig. 4). If the amino group is substituted in the manner suggested by Busch, Patrascanu, and Weber (1934), the chromophores will be insulated and their extinctions additive : therefore it will be impossible to determine from data such as those in Figure 4 the distribution between uni- and bisubstituted products for initial molar ratios $>\frac{1}{2}$. If, on the other hand, bifunctionality were to lead to a conjugated or cumulated system the data of Figure 4 would suggest that the group reacted in an *exclusively* bifunctional way, for if both uni- and bi-derivatives were present, their extinctions would not be additive, which would result in deviation from the constancy of total extinction at molar ratios $>\frac{1}{2}$. In addition, with different products more than one major absorption band would be expected in the electronic

spectrum. The Busch mechanism appears the more plausible however, and if this is accepted the distribution of the substituent groups among the excess amino acid molecules will not influence the extinction greatly. We shall therefore proceed as if the amino group were in fact exclusively bifunctional.*

Absorption spectra produced by tryptophane on reaction with diazotized sulphanilic acid are shown in Figure 5. By analogy with the behaviour of glycine, the absorption band at *c.* 360 mμ can be assigned to an amino substituent, which is insulated from the indole group. The maximum in the region of 270 mμ can be attributed partly to unreacted *p*-diazobenzenesulphonic

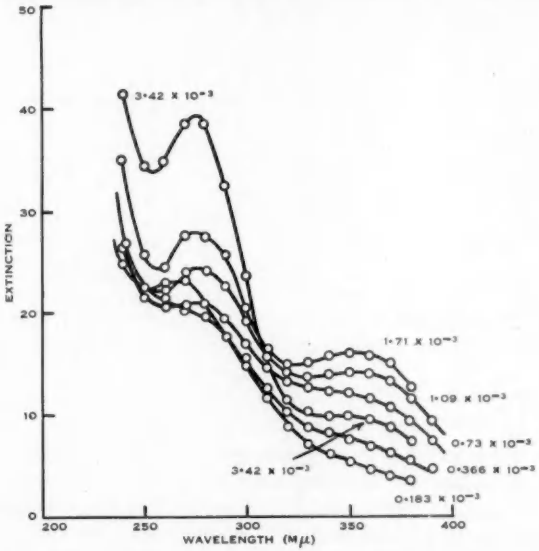

Fig. 5.—Absorption spectra produced by tryptophane upon reaction with diazotized sulphanilic acid. Figures beside each curve are initial molar concentrations of tryptophane.

acid (low initial tryptophane concentrations) and partly to unreacted tryptophane (high initial concentrations) which absorb at *c.* 267 and *c.* 275 mμ respectively. The dependence of the observed extinction, E, on the initial molar ratio (Fig. 6) shows a minimum near $a/b=1/3$ (using the nomenclature of Section II) suggesting that the tryptophane molecule can accept a third diazonium group, and that an absorption band near 270 mμ persists in the indole derivatives, AB and B_2AB.

Estimates of the molar extinction coefficients, ε, for the various components of the final system may be made from the observed relationships between extinction and initial molar ratio. As shown in Figures 1 and 2, the relationship between C/b and a/b is linear in certain ranges of a/b for B and B_nAB (regardless

* This conforms to the assumption made in the last sentence of Section I.

of the value of K) and approximately so for A at high values of a/b. In those ranges of initial molar ratio in which only one component is contributing to the change in observed extinction with a/b, the slope of the curve

$$\frac{\Delta E}{\Delta(a/b)} = \frac{\Delta(C.\varepsilon)}{\Delta(a/b)} \quad \text{(Beer's law)}$$

$$= b.\varepsilon \frac{\Delta(C/b)}{\Delta(a/b)},$$

or

$$\varepsilon = \frac{\text{Slope (observed)}}{b.\Delta(C/b)/\Delta(a/b)}.$$

The value of b is known and $\Delta(C/b)/\Delta(a/b)$ can be obtained from Figures 1 or 2, the Δ's all referring to the same a/b range.

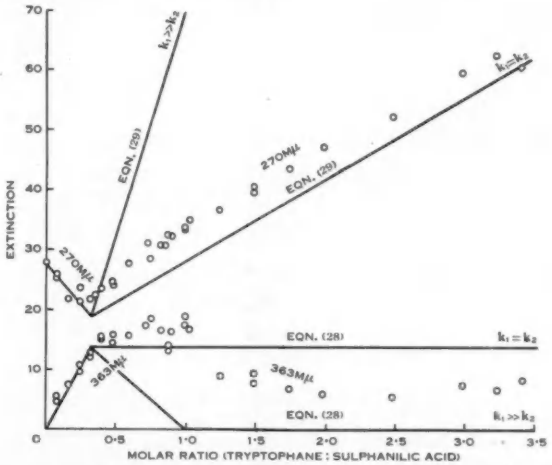

Fig. 6.—Dependence of final concentrations at 270 and 363 mμ upon initial tryptophane concentration.

Values of ε calculated by this method were approximate multiples of 6000 and the following values were used in the subsequent calculations:

(1) $\varepsilon^{363}_{B_2AB} = \varepsilon^{363}_{B_2A} \doteqdot 18{,}000$: This was calculated for B_2AB from the initial slope of the E_{363} curve (experimental) of Figure 6, with $a/b < 1/3$ (and $C_{B_2A} = 0$). In the molecules B_2AB and B_2A the B_2 structure is assumed to have the same ε, being electronically insulated from the other absorbing structures.

(2) $\varepsilon^{270}_{A} = \varepsilon^{270}_{B_2A} \doteqdot 6000$: This was calculated from A from the slope of the E_{270} curve at high molar ratio. (Other observations, with $b=0$ and $a = 0{\cdot}46 \times 10^{-3}$ mole/l confirmed this value.)

(3) $\varepsilon^{270}_{B} \doteqdot 12{,}000$: This was calculated from the value of E_{270} when $a=0$ (and $C_A = C_{AB} = C_{B_2A} = C_{B_2AB} = 0$).

(4) $\varepsilon^{270}_{B_2AB}=\varepsilon^{270}_{AB}\div 24{,}000$: This was calculated for B_2AB from the initial slope of the E_{270} curve, with $a/b<1/3$ (and $C_A=C_{AB}=C_{B_2A}=0$) so that $E_{270}=\varepsilon^{270}_{B}C_B+\varepsilon^{270}_{B_2AB}C_{B_2AB}$.

Also ε^{363}_{AB}, ε^{363}_{A}, and ε^{363}_{B} may be taken as zero, since AB, A, and B do not contain the B_2 structure which absorbs at 363 mμ.

In general,

$$E_{363}=E_{B_2A}+E_{B_2AB},$$

and

$$E_{270}=E_A+E_B+E_{AB}+E_{B_2A}+E_{B_2AB},$$

so that

$$E_{363}=b\varepsilon^{363}_{B_2A}[(C_{B_2A}+C_{B_2AB})/b], \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (25)$$

and

$$E_{270}=b\varepsilon^{270}_{A}[(C_A+2C_B+4C_{AB}+C_{B_2A}+4C_{B_2AB})/b]. \quad \ldots\ldots \quad (26)$$

When $K=1$ and $a/b>1/3$ we get

$$\frac{C_A}{a}=\left(1-\frac{b}{3a}\right)^2, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (27)$$

and from equations (**26**) to (**28**) of the 1953 paper

$$C_{AB}/a=C_{B_2A}/a=(C_A/a)^{\frac{1}{2}}-C_A/a,$$

and

$$C_{B_2AB}/a=1-2(C_A/a)^{\frac{1}{2}}+C_A/a.$$

From the above we can calculate C_A/b, C_{AB}/b, C_{B_2AB}/b, and C_{B_2AB}/b for various values of a/b. Equations (**25**) and (**26**) become

$$E_{363}=41\cdot6[(C_{B_2A}+C_{B_2AB})/b], \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (28)$$

and

$$E_{270}=13\cdot9[(C_A+5C_{AB}+4C_{B_2AB})/b]. \quad \ldots\ldots\ldots\ldots \quad (29)$$

In Figure 6 the calculated curves for E_{363} and E_{270} are drawn for $k_1\gg k_2$ and $k_1=k_2$. Comparison with the experimental data in the same figure shows that the theory provides at least a partial interpretation of what at first sight appears to be a most complex dependence of extinction, at the two absorption maxima, upon the initial molar ratio. Certain discrepancies are apparent, particularly in the E_{363} data between molar ratios of 1/3 and 1. These will not be discussed here in the light of the limited data available, which are given merely to illustrate the method of obtaining information on the relative reactivity of the various positions in the molecule. It is fairly evident that the results are in accordance with a higher velocity constant for reaction at the indole group than for reaction at the amino group.

As the nitrogen atom of the amino group is insulated from the indole structure in the tryptophane molecule there is some justification for assuming that the velocity constant for reaction at one of these positions will not be greatly affected by substitution at the other, that is, that $k_1=k_4$ and $k_2=k_3$, as in the theoretical treatment.

IV. Acknowledgments

The assistance of Miss Dallas Fraser and Mr. K. J. Harrington is acknowledged with thanks. The author also wishes to thank Dr. E. J. Williams and Mr. R. S. T. Kingston for helpful comments on the manuscript.

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THE RANGE–ENERGY RELATION FOR α–RECOIL ATOMS

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Summary

The ranges of ThC″ α-recoil atoms have been redetermined at pressures of hydrogen, argon, and air from 10^{-3} to 30 mm, by collection on a silver plate mounted 3 mm from a platinum source plate. The collector was maintained at a positive potential of 300 V to prevent collection of ions which migrate by diffusion. A similar method is used for RaA, with a (gaseous) radon source.

Previous work is critically discussed in terms of the corrections necessary to allow for (i) geometrical features, and (ii) range straggling. Values of the mean range calculated both from previous work and from the present work are plotted against the recoil energy. Both the Flügge-Zimens recoil-range equation and the stopping power equation are shown to be unsatisfactory, and to rest on arguments which are subject to serious objection on both experimental and theoretical grounds. The recent Bohr treatment of collisions between recoil atoms and gaseous atoms is in much better agreement with the unobjectionable experimental data available.

I. Introduction

The currently accepted range-energy relation for α-recoil atoms is that of Flügge and Zimens (1939), which is based on the range measurements of previous workers. It is of the form

$$R=CE^2, \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where C is a constant obtained from the plot of the logarithm of the range R against the logarithm of the recoil energy E. The relative stopping powers of different substances are given, according to Flügge and Zimens, by the relation

$$R=\frac{c}{d}\left(\frac{A}{B}\right), \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where d is the density of the stopping medium, A its atomic weight, and B is the stopping power for α-particles. For materials containing more than one element, a mean value of A/B is used. The constant c depends on the recoil energy, and is different from C.

Since this work was done, Bohr (1948) has discussed the range-energy relation in terms of modern theories of stopping of atomic particles. Assuming adiabatic collisions between the recoil atom and the atoms which it hits, Bohr obtains by classical mechanics the relation

$$R_0=\frac{m_e E}{\pi N \hbar^2 Z_1^{\frac{1}{2}} Z_2}, \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

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where m_e is the electron mass; N is the number of atoms of stopping material/cm^3; Z_1 and Z_2 are the charges on the nuclei of the recoil atom and the struck atom respectively, and R_0 is the mean range of the recoil atom.

It will be seen that equations (1) and (2) give a different value of the range in a particular stopping material from equation (3), and different variations with energy, and type of stopping material.

We have reassessed the earlier work in the light of equation (3) and have redetermined the ranges of ThC″ and RaA. To allow accurate assessment of the mean range, and the straggling, the range determinations were made by allowing the recoil atoms to traverse a known distance from a source to a metal collecting plate through a gaseous stopping medium, the pressure of which could be varied, and accurately measured.

II. Experimental

(*a*) *Apparatus*

The source of recoil atoms was a circular piece of platinum foil, of 2·30 cm diameter, on which was deposited an equilibrium mixture of ThB, ThC, ThC′, and ThC″. The ThC″ recoil fragments were collected on a metal plate with an exposed circular area of 25·6 mm diameter, sandwiched between two rings of "Perspex" as insulators. A potential difference could be applied, if necessary, between the source and collector, which were 2·90 mm apart. This assembly was enclosed under a glass bell jar, sealed from the atmosphere with a rubber gasket. It could be evacuated to a pressure of 10^{-3} mm mercury, and there was provision for admitting gases which had been filtered through cotton wool and dried over calcium chloride and silica gel.

The same apparatus was used for determination of the recoil range of RaA, except the plates were 1·55 mm apart. Radon mixed with the stopping gas was allowed to circulate freely between the plates. The recoil range was determined from the yield of RaA obtained on one plate, the back of which was covered with a lacquer. After exposure, the lacquer was removed so that any recoil atoms collected on this face would not be detected.

(*b*) *Source Preparation*

(i) *ThB/C Mixture.*—A negative potential of about 200 V was applied to the platinum foil used for the source plate, which was exposed for periods up to 3 days to a source of thoron, usually thorium hydroxide.

(ii) *Radon.*—Radon was collected in a 300-ml flask, attached by cone-socket joint to a vacuum line, in which was a vessel containing a 4-mc source of radium chloride. The assembly was evacuated to a pressure of about 0·1 mm before collection, which was continued for periods up to 4 hr. The flask was closed with a greased tap, disconnected, and reconnected to an apparatus in which the recoil experiments were performed. To obtain an estimate of the relative amounts of radon collected, a potential of 200 V was maintained between two plates held in the vacuum line during collection of the radon. The activity obtained on the positive electrode after 30 min was determined.

(c) *Method*

(i) *ThC″ Recoil.*—After measurement of the activity of the ThB/C source, it was mounted opposite the collector plate, and the apparatus pumped down to 10^{-3} mm. The desired gas was then introduced, and the pressure recorded. The desired potential difference between the plates was supplied from a 2000 V stabilized power supply. After a known time, the potential difference was removed, air was admitted, and the activity of the collector plate was measured with an end-windowed Geiger counter. The activity decayed with a 3·10 min half-life (ThC″), which could be extrapolated to zero time, taken as the time when the potential difference between the plates was removed. The yield of ThC″ recoil atoms was taken as the zero time activity of the collector (600–900 counts/min with pressures below 0·1 mm) expressed as the percentage of the total source activity at the commencement of the experiment.

Stopping gases were prepared as follows. Cylinder hydrogen was introduced through a heated palladium thimble. Both cylinder argon and air were dried over calcium chloride. Mass spectrometric analysis of argon gave the following percentages: A, 99·9; N_2, 0·1; Ne, Kr, Xe, and O_2 all less than 0·02.

(ii) *Radon Recoil.*—After evacuation of the apparatus (10^{-3} mm Hg), a mixture of air and radon was admitted at a known pressure. A potential difference was applied between the plates, and the assembly left for 3 hr. The activity of the collector (positive) electrode was found to rise initially, due to growth of RaB and RaC, and then to decay with a half-life of 27 min (RaB). The recoil yield was taken as the maximum activity of the decay curve (obtained after ~16 min), and was expressed as the percentage of the maximum activity of a similar curve obtained from the material collected on the calibration plate while radon collection was in progress.

III. Results

The following preliminary experiments were performed as a check to ensure that the recoil yield measurements were meaningful.

(a) *Collection Time*

As expected, the recoil yield of ThC″ under constant conditions was found to grow exponentially with time, reaching a value which was constant within experimental error after about 19 min (~6 ThC″ half-lives). A standard collection time of 20 min was therefore used in all experiments.

(b) *Applied Potential*

The yield of recoil atoms depends critically on the applied potential difference between the collector and source. With the collector negative (>200 V), high yields can be obtained even at atmospheric pressure, whereas with the opposite polarity much lower yields are obtained. For ThC″ in air with a positive potential on the collector the yields become negligible at about 25 mm mercury. With zero-potential the yields are similar to but rather higher than those obtained with a positively charged collector. These observations are consistent with the known fact that α-recoil atoms of the heavy metals are

positively charged. They arrive at the collector plate either directly by recoil or—if the pressure is sufficiently high to cause them to be reduced to thermal velocities in the gas space—by diffusion. With a negatively charged collector the diffusing atoms are dragged out of the gas, whereas a positively charged collector prevents such low energy recoil atoms from being collected. For this reason, a positively charged plate was used throughout the range measurements.

The magnitude of the retarding potential necessary to prevent diffusion was investigated, with the results shown in Figure 1. A retarding potential of 300 V was used thereafter.

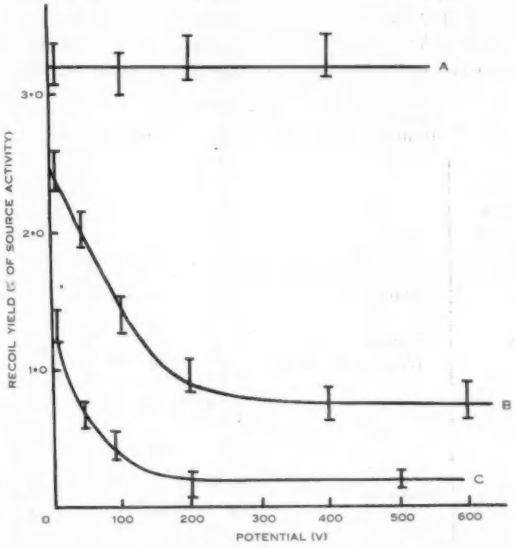

Fig. 1.—Variation of yield with positive collector potential.
A, 0·2 mm Hg; *B*, 14 mm Hg; *C*, 21 mm Hg.

(c) *Estimation of ThC″ Activity of Source*

Three of the emitters present in the source, namely, ThB, ThC, and ThC″, are β-active. The data are given in Table 1 (National Bureau of Standards 1950). An aluminium absorption curve of the source was determined, but it was not possible to distinguish clearly any of the β-particles present. The following procedure was adopted for estimating the ThC″ activity.

At absorber thicknesses above 300 mg/cm² the main contributions to the measured activity are from the 1·79 and 2·25 MeV β-particles. There will also be much smaller contributions from the 1·55, 1·51, and 1·42 MeV particles. Aluminium absorption curves on the same apparatus were available for some β-emitters in this energy region, and in particular for two β-emitters of approximately the same energy as the two principal particles (1·79 and 2·25 MeV),

namely, ^{32}P (1·70 MeV) and UX_2 (2·32 MeV) (Duncan and Thomas 1957). These show that at 300 mg/cm² of aluminium the ^{32}P activity has fallen to 0·142 of its extrapolated value at zero thickness and the UX_2 to 0·219. Similarly, extrapolation between other absorption curves gives the corresponding values for the other β-particles involved. The activity of the ThC″ in the source measured at zero thickness will then be a fraction

$$f=\frac{1}{\Sigma\nu\gamma}\times\frac{\text{measured activity at 300 mg/cm}^2}{\text{measured activity at zero thickness}}$$

of the measured activity—γ is the fractional activity for a particular β-particle at 300 mg/cm² (e.g. for the 2·25 MeV β-particle $\gamma=0\cdot218$); ν is the frequency factor which takes into account the percentage contribution of each particle to the decay scheme and also the ThC branching ratio (e.g. for the 2·25 MeV

TABLE 1

β-DECAY ENERGIES OF ThB, ThC, AND ThC″

Emitter	Reference	Maximum β-Energy (MeV)	Frequency (%)
ThB	National Bureau of Standards (1950)	0·355	88
		0·59	12
ThC	National Bureau of Standards (1950)	2·25	~55
		1·55	~13
		1·42	~14
		0·65	~5
		0·45	~9
		0·05	~4
ThC″	Richardson (1948)	1·79	50–60
		1·51	20–30
		1·28	20

β-particle $\nu=0\cdot55\times2$). The summation is taken over the principal β-particles involved in the decay. A series of values of f was obtained at absorber thicknesses between 200 and 500 mg/cm². The values below 200 mg/cm² still contain contributions from softer β-particles, and above 450 mg/cm² there is considerable experimental scatter due to low counting rates. Basing the assessment on measurements between these values, we obtain $f=13\cdot3\pm1\cdot0$ per cent. The 1 per cent. error quoted includes both experimental inaccuracies and uncertainties in the decay scheme of ThC″ (Richardson 1948).

(*d*) *Collection Efficiency*

It is shown below that, with the geometry used, about 67·5 per cent. of the recoil fragments emitted from the plate will strike the collector, of which only 50 per cent. will emit β-particles in the direction of the detector when the activity is being measured. If collection of ThC″ recoil atoms is 100 per cent. efficient, the apparent recoil yield will be expected to be $Y=0\cdot68\,f/2$, that is, a value of

$4 \cdot 5 \pm 0 \cdot 3$ per cent. of the activity of the source measured under the same geometrical conditions as the collector. This compares with an experimentally observed value of $3 \cdot 20 \pm 0 \cdot 05$ per cent. The above values give a collection

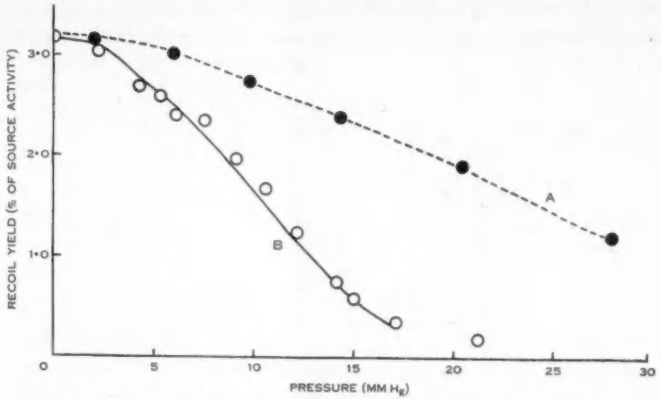

Fig. 2.—Range of ThC′ in air. Yield *versus* air pressure. Curve *A*, no retarding potential; points ○, retarding potential +300 V; full curve (*B*), calculated as in Section III for $R_0 = 0 \cdot 067$ mm at 760 mm pressure.

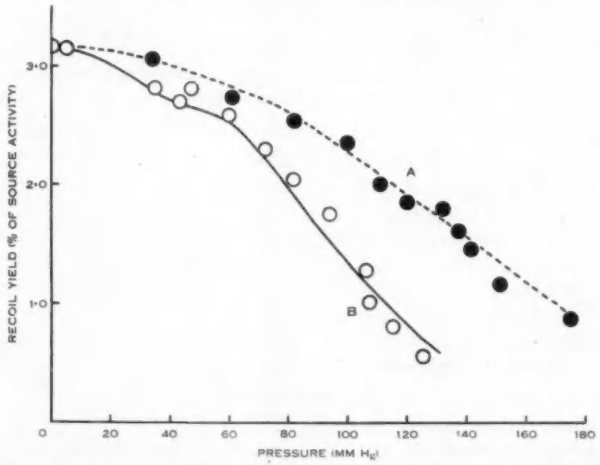

Fig. 3.—Range of ThC′ in hydrogen. Yield *versus* hydrogen pressure. Curve *A*, no retarding potential; points ○ retarding potential +300 V; full curve *B*, calculated as in Section III, for $R_0 = 0 \cdot 56$ mm at 760 mm pressure.

efficiency of 67–76 per cent. We obtain the same yield on different collecting surfaces (silver, copper, mica), which confirms that the collection efficiency is high (see also Kovarik 1912).

(e) *Collection of ThC″ and RaA Atoms*

The experimental values for the yield of ThC″ atoms are plotted in Figures 2, 3, and 4 as a function of the pressure. Those for RaA are shown in Figure 5. In Figures 2, 3, and 4 the experimental points are accurate to better than 0·15 in the percentage yield. In Figure 5, the error is less than 0·06 in the fraction collected.

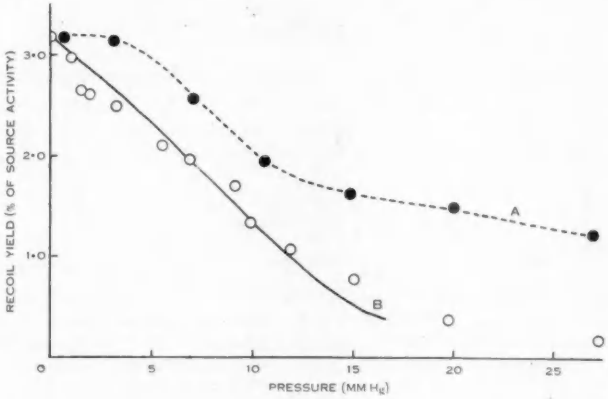

Fig. 4.—Range of ThC″ in argon. Yield *versus* argon pressure. Curve *A*, no retarding potential; points ○ retarding potential +300 V; full curve *B*, calculated as in Section III, for $R_0=0{\cdot}059$ mm at 760 mm pressure.

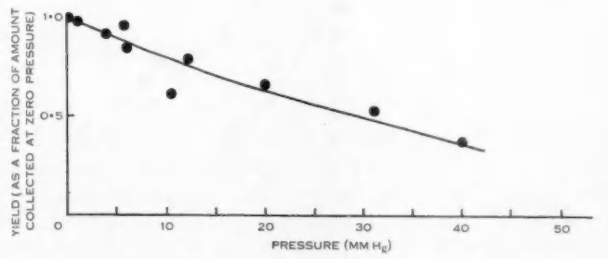

Fig. 5.—Range of RaA in air. Yield *versus* air pressure. Experimental points determined with a retarding potential of +300 V. The curve is calculated as in Section III; for $R_0=0{\cdot}060$ mm at 760 mm pressure.

IV. Treatment of Results

The shape of the experimental range-pressure curve depends not only on the mean range, but also on the range straggling, and on the geometry of the apparatus used. To evaluate the mean range, therefore, some consideration must be given to the other two factors.

(*a*) *Geometrical Correction*

Consider a point on the surface of the source plate. The number of recoil atoms emitted from this point, which are collected, will be contained in the solid angle subtended by the circle of intersection of a sphere, of radius equal to the range, with the plane of the collector. But not all of these will be collected, since the collector plate is not of infinite area. Three cases are possible, shown in Figure 6.

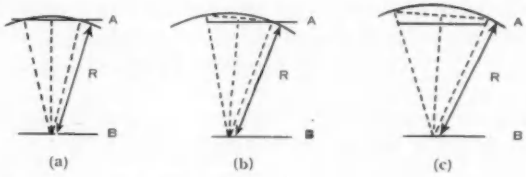

Fig. 6.—Three possible types of collection geometry. *R*, range ; *A*, collector plate ; *B*, source plate.

The fraction F of recoil atoms emitted by the source plate which are received by the collector may be shown (Barton 1956, personal communication) to be

$$F=\frac{1}{\pi A^2}\int_0^A x\mathrm{d}x\iint \sin\theta\,.\,\mathrm{d}\varphi\,.\,\mathrm{d}\theta, \qquad \ldots\ldots\ldots\ (4)$$

where A is the ratio of the radius of the source plate to the plate separation ; x is the ratio of the distance of an emitting point from the centre of the source plate to the plate separation ; θ and φ are the zenith and azimuth angles of a spherical polar coordinate system with the emitting particle as pole.

The integration is extended to include all collected particles. Figure 7 shows the plot of F against R, the range of the recoil atoms. The maximum value is 0·675, that is, at low gas pressure (large range) 67·5 per cent. of the atoms leaving the source is collected. A rough experimental check of this value was made using a UX_1 β-active source of known activity of the same area as the source of α-recoil atoms. A large-windowed Geiger counter was substituted for the collector plate. With this system, a geometrical efficiency of 58 per cent. was obtained. In view of the errors from source non-uniformity, β-particle scattering, counter dead space, etc. likely to arise in this case, the calculated value of 67·5 per cent. was used. But it should be emphasized that the absolute values of F are unimportant. The significant quantity in evaluating the theoretical plots of Figures 2–4 above is the ratio of F for any given range (or pressure) to the value of F for infinite range (zero pressure). This is accurately given by Figure 7.

(*b*) *Range Straggling*

According to Bohr, the straggling of particles about the mean range R_0 may be described by means of the normal curve

$$W(R)=\frac{1}{\sqrt{(2\pi)}s}\exp\left[-\frac{(R-R_0)^2}{2s^2}\right], \qquad \ldots\ldots\ldots\ (5)$$

where s, the standard deviation of the normal distribution, is given by

$$\frac{s^2}{R_0^2}=\frac{4}{3}\frac{m_2}{m_1}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

$W(R)$ is the probability that a particle in a group of particles with mean range R_0 will have a range between R and $R+\mathrm{d}R$; m_1 and m_2 are the masses of the recoil atom and the atom of the stopping material.

Also, the number of recoil atoms collected, having a range R, is

$$\frac{1}{Y_0}\left(\frac{\mathrm{d}Y}{\mathrm{d}R}\right)=\frac{F}{\sqrt{(2\pi)}s}\exp\left[-\frac{(R-R_0)^2}{2s^2}\right], \quad \ldots\ldots\ldots \quad (7)$$

where Y_0 and Y are respectively the numbers of atoms leaving the source and being collected, and F is the geometrical factor (eqn. (4)).

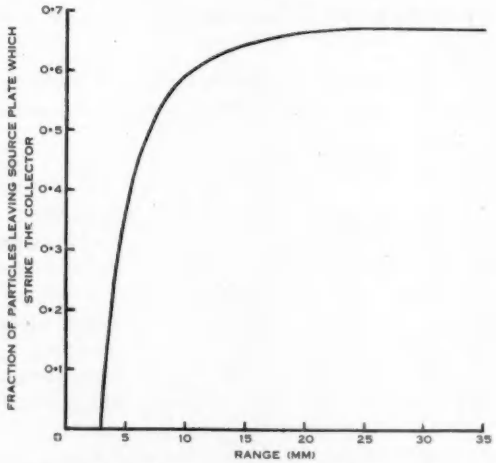

Fig. 7.—Effect of geometry on yield for different values of the range.

The number of recoil atoms collected is given by the integral of this function from $R=2\cdot90$ mm to $R=\infty$. This was evaluated graphically, with the results shown in Figures 2–4.

(c) *Calculation of Mean Range*

We are now ready to treat the experimental results. Two methods were used.

Method 1.—For a given stopping gas and a given recoil atom the mean range R_0 is k/p, where p is the pressure and k is a constant. In this method, particular values of k are used to estimate corresponding values of R_0 at different pressures. These are inserted in equation (7) to obtain curves of yield v. pressure. These curves, derived from theory only, are compared with experiment, and the value of k giving the best fit is taken as correct.

Method 2.—Alternatively, we may use a particular value of R_0 to calculate the yield expected. Combining this with the measured value of the pressure at this particular value of Y, values of k may be obtained along the curve. The values should be the same for a particular curve.

In these calculations it is necessary to choose some value of s. The value was taken as that given by the theoretical relation of Bohr (eqn. (5)) for the following reasons:

(i) With one exception (Wood 1913) it is in agreement with previously determined straggling curves (see Table 2).

(ii) Range values calculated using this value of s are in excellent agreement with our experimental values.

(iii) There are no other values of s (theoretical or empirical) available.

In fact, the shape of the yield-pressure curves is changed very little by two-threefold variations in s.

(*d*) *Radon Recoil*

Similar methods were used for this system, but since the source is uniformly distributed between the plates the geometrical correction is different. We also make the simplifying assumption that the fraction F of atoms of range R collected is given by

$$F=\frac{1}{L}\int\left(1-\frac{h}{R}\right)\mathrm{d}h$$

$$=1-L/2R \quad \text{between the limits 0 and } L, \text{ for } R>L$$

$$=R/2L \quad \text{between the limits 0 and } R, \text{ for } R<L,$$

where L=distance between the source and collector plate (=1·55 mm) and h is the distance from the collector of a parallel plane of radon atoms. The calculation is then identical with that discussed above.

V. Discussion

(*a*) *Theoretical Treatment of Data*

(i) *Present Work.*—The theoretical recoil yield-pressure curves calculated for ThC″ from the Bohr expression, as outlined above, are shown in Figures 2–4. It will be seen that good agreement is obtained for all three gases. In particular, the calculation requires that the curves shall be distorted from the simple sigmoid shape, due to geometrical factors. Our experimental data show some, although not conclusive, evidence of this.

The results of range measurements of RaA in air are shown in Figure 5, together with the theoretical curve. Good agreement is again obtained.

(ii) *Previous Work.*—In Table 2, the experimental values of the range obtained in the present work are quoted alongside those of earlier work. It

TABLE 2

RANGE DETERMINATIONS

Recoil Atom	Recoil Energy (keV)	Experimental Range (mm at 760 mm pressure)	Experimental Features	Treatment of Results	Corrected Range (mm at 760 mm pressure)	Calculated Range (eqn. (3))	Reference
Rn in air	86	0·064	Calculated from emanating power of glass rods of known dimensions	Rejected	—	0·049	Flügge and Zimens (1939)
RaG in air	100	0·090	Extrapolated value. Results not given in detail	Rejected	—	0·058	Bianu and Wertenstein (1912)
RaA in air	101	Figure 5		As Section III	0·064 ± 0·003*	0·059	Present work
Tn in air	103	0·083	Calculated from emanating power of glass rods	Rejected	—	0·059	Flügge and Zimens (1939)
Tn in barium stearate	103	940 Å	Extrapolated	As Section III Use equation (3) to give range in air	960 Å in Ba stearate 0·057 in air*	0·059	Gregory, Hill, and Moorbath (1952)
RaB in air	112	0·14	Collimated beam and collector plate. Straggling agrees well with Bohr theory	As Section III	0·092*	0·065	Wertenstein (1914)
RaB in H_2	112	0·83	The same. Straggling agreement reasonable	As Section III	0·72	0·46	Wertenstein (1914)
ThC″ in air	116	0·13	No retarding potential. No geometrical correction	Rejected	—	0·067	Chamié (1934*a*, 1934*b*)
ThC″ in air	116	0·12	Retarding potential unknown. Extrapolated range. Larger straggling than expected theoretically	Assume point source at centre of hemispherical collector. Treat as Section III	0·070*	0·067	Wood (1913)

* These values were used in the range-energy plot (Fig. 8).

TABLE 2 (*Continued*)

TABLE 2 (*Continued*)

Recoil Atom	Recoil Energy (keV)	Experimental Range (mm at 760 mm pressure)	Experimental Features	Treatment of Results	Corrected Range (mm at 760 mm pressure)	Calculated Range (eqn. (3))	Reference
ThC″ in air	116	Figure 2		As Section III	0·067 ± 0·002*	0·067	Present work
ThC″ in H_2	116	Figure 3		As Section III	0·56 ± 0·01	0·48	Present work
ThC″ in H_2	116	0·55	Extrapolated, No experimental results given	As Section III. Straggling estimated from (i) equation (4)	0·47		Wood (1913)
				(ii) Wood's data for air	0·50	0·48	
ThC″ in H_2	116	0·50–0·68	Uncertainty in dead space of spark counter	Rejected	—	0·48	Kolhörster (1920)
ThC″ in argon	116	Figure 4	Straggling correction not so good as for ThC″ in air and H_2	As Section III	0·059 ± 0·005	0·054	Present work
AcC″ in air	126	0·12	Extrapolated. Insufficient experimental data given	Rejected	—	0·072	Wood (1913)
AcC″ in air	126	0·126	Extrapolated. Ionization chamber detector	Rejected	—	0·072	Wood (1913)
AcC″ in H_2	126	0·52	Extrapolated. Insufficient experimental data given	Rejected	—	0·52	Wood (1913)
AcC″ in H_2	126	0·55	Extrapolated. Ionization chamber detector. No experimental data given	Rejected	—	0·52	Wood (1913)
AcA in a small quantity of air saturated with water vapour	127	0·07–0·08	Cloud chamber. Straggling curve in excellent agreement with Bohr theory	No treatment necessary	If stopped by air 0·07–0·08*; if stopped by water vapour 0·05–0·06	0·072	Joliot (1934)

* These values were used in the range-energy plot (Fig. 8).

TABLE 2 (*Continued*)

Recoil Atom	Recoil Energy (keV)	Experimental Range (mm at 760 mm pressure)	Experimental Features	Treatment of Results	Corrected Range (mm at 760 mm pressure)	Calculated Range (eqn. (3))	Reference
AcA in air	127	0·092	Collector plate. Similar to RaA technique in this paper	As Section III. Approximation in estimation of geometrical correction	0·08–0·09*	0·072	McKeehan (1917)
RaD in air	146	0·17	Extrapolated. Insufficient experimental data	Rejected	—	0·084	Bianu and Wertenstein (1912)
ThD in air	169	0·175	Extrapolated. Ionization chamber detector. Insufficient experimental data	Rejected	—	0·097	Wood (1913)
ThD in H_2	169	0·74	The same	Rejected	—	0·70	Wood (1913)
ThD in H_2	169	0·87–1·12	Uncertainties in dead space of spark counter	Rejected	—	0·70	Kolhörster (1920)

* These values were used in the range-energy plot (Fig. 8).

will be noticed that there is wide disagreement. This is due to unsatisfactory features in the earlier work arising for one of the following reasons :

(1) No retarding potential has been applied, to eliminate diffusion.

(2) An extrapolated range has been quoted, whereas our results refer to a mean range. It is possible to estimate the mean range from the extrapolated range, provided the relative contributions of straggling and geometry are known. If not, no correction can be made.

In Table 2, corrections to the previously determined ranges have been made where possible. The remaining data have been discarded, for the reasons indicated. The recoil energy E_R quoted is calculated from α-particle decay energy E_α using the relation

$$E_R = 4E_\alpha/M,$$

where M is the mass of the recoiling atom.

(*b*) *The Range-Energy Relationship*

The experimental relation of Flügge and Zimens (eqn. (**1**)) is based on uncorrected experimental range values quoted in Table 2. From the foregoing discussion it will be seen that this relation is not valid, for the following reasons :

(i) Extrapolated ranges have been used (except for thoron and radon) and no correction has been made for different experimental arrangements—in spite of the fact that a wide variety of different geometrical arrangements was used.

(ii) At least one experimental curve shows straggling which is much larger than predicted by equation (**4**), whereas other systems are in good agreement (see Table 2).

(iii) A number of different detecting arrangements were used, some of which have a large zero error. In particular, it would appear from the work of Madsen (1945) that an ionization detector is not sensitive to recoil atoms over the last quarter of their range.

In Figure 8 the corrected range values quoted in Table 2 are plotted as a function of the recoil energy, and compared with the theoretical plots expected on the Flügge-Zimens equation (**1**) and on the Bohr treatment (eqn. (**3**)). The results favour a range proportional to the first power of the energy, rather than a second power. We therefore must conclude that the evidence in favour of equation (**1**) is inadequate.

(*c*) *The Mass Stopping Equation*

The Flügge-Zimens mass stopping relation (eqn. (**2**)) depends on the assumption that the atomic stopping power of a substance for recoil atoms is the same as that for α-particles. The evidence cited for this is that the range in hydrogen is approximately 0·2 of that in air, compared with a fraction 0·21 for a 6 MeV α-particle. But, as before, comparison was made using extrapolated values of the range. Because of the large differences in the straggling in air and in hydrogen (see Figs. 2 and 3), the fraction is quite different if mean

ranges are used, for ThC″ it is 0·13. This divergence from the α-particle range ratio is not surprising since the mechanism of stopping α-particles differs considerably from that for recoil atoms. In particular, almost all the energy loss by recoil atoms occurs in elastic collisions with gas molecules. A relatively small loss of energy is caused by ionizing collisions which is the main feature of α-particle collisions with gases. This has been known since the early work on recoil ions (Wertenstein 1914) and has since been confirmed (Madsen 1945). Bohr made allowance for this feature in deriving equation (3), which is based on a treatment

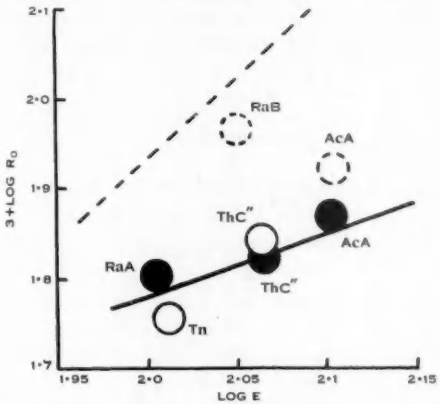

Fig. 8.—Range-energy relationship in air. (R_0 in mm at 760 mm pressure; recoil energy (E) in keV). Full points ● are the most reliable data available. RaA, ThC″, present work; AcA (Joliot 1934), but relative quantities of water vapour and air are unknown. Points ○ are less reliable. Tn (Gregory 1952) was determined in *solid* barium stearate. ThC″ (Wood 1913)—the straggling curve is not in good agreement with theory. Dotted points ○ are the least reliable. RaB (Wertenstein 1914)—all range values quoted are much higher than the corresponding figures obtained by us, and others. AcA (McKeehan 1917)—an approximate method had to be used by us to estimate the geometrical correction. The full line is calculated from Bohr theory (eqn. (3)). The dotted line is that given by the Flügge-Zimens stopping power equation (eqn. (1)).

of purely elastic collisions. However, the accuracy with which the Bohr relation may be applied is limited by the uncertainty in the value to be taken for the collision diameter.

The ranges of ThC″ recoil atoms in different gases may be calculated both from equations (1) and (2) (assuming the Flügge-Zimens value of $C=8\cdot7$ mm/MeV²) and from equation (3) (see Table 3). It will be seen that the values quoted on the Bohr treatment are reasonably close, which theory is to be preferred to the Flügge-Zimens equation.

(*d*) *Conclusion*

From both the range-energy relation in air, and from the stopping power of different materials for ThC″ recoil atoms, we conclude that (i) the evidence

for the empirical relationships of Flügge and Zimens is inadequate; (ii) there is much in favour and nothing in obvious disagreement with the Bohr formula, which is in more satisfactory agreement with the experimental evidence at present available.

TABLE 3

CALCULATED RECOIL RANGES FOR ThC″ RECOIL ATOMS (MM AT 760 MM PRESSURE)

Stopping Material	Mean Expt. Value	Flügge-Zimens*	Bohr
Hydrogen	0·56	0·687	0·48
Argon	0·059	0·128	0·054
Air	0·067	0·117	0·067

* The Flügge-Zimens treatment is applied to extrapolated ranges and therefore a direct comparison with the experimental mean values is not possible. Conversely, a meaningful extrapolated range cannot be obtained from our results since it is a function of the geometry. Our geometry was different from several of the methods used to obtain points in the Flügge-Zimens plot.

VI. ACKNOWLEDGMENTS

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THE IONIZATION CONSTANTS OF *p*-AMINOBENZOIC ACID IN AQUEOUS SOLUTION AT 25 °C

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Summary

The ionization constants of benzoic acid and of four esters of *p*-aminobenzoic acid, as well as the two ionization constants of *p*-aminobenzoic acid itself, have been determined by spectrophotometric measurements. The extent of zwitterion formation in *p*-aminobenzoic acid is discussed.

I. Introduction

The ionization constant of a weak acid can be determined with considerable accuracy by measuring the ultraviolet absorption due to the acid in a suitable buffer whose pH value is known accurately, provided there is a wavelength in the spectrum at which the absorption due to the neutral undissociated molecule and that due to the charged anion are markedly different. The method has been applied to *p*-nitrophenol (Robinson and Biggs 1955), to a number of phenol derivatives (Biggs 1956), to vanillin and two of its isomers (Robinson and Kiang 1955), and to vanillylamine (Robinson and Kiang 1956).

Table 1

IONIZATION CONSTANTS OF *p*-AMINOBENZOIC ACID

References	Temp. (°C)	pK_1	pK_2
Johnston (1906)	25	2·41	4·92
Hölmberg (1908)	25	—	4·94
Pring (1923)	20	2·35	—
Myrbäck (1926)	25	2·20	—
Albert and Goldacre (1942) ..	20	2·66	4·82
Klotz and Gruen (1945) ..	25–27	2·50	—
Schwarzenbach, Willi, and Bach (1947)	20	2·537	4·699
Present work	25	2·45	4·85

p-Aminobenzoic acid is a substance of considerable biochemical and pharmacological interest, being the parent of such substances as procaine (the β-diethylaminoethyl ester) and benzocaine (the ethyl ester). It is often quoted as an example of incomplete zwitterion formation, about 12 per cent. being thought to be present as $NH_3^+.C_6H_4COO^-$, the remaining 88 per cent. being present as

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$NH_2.C_6H_4.COOH$, in contrast to glycine in which the zwitterion predominates to an overwhelming extent. However, there is some doubt as to the true values of its ionization constants as Table 1 shows.

Therefore, we have measured these two constants by the spectrophotometric method, as well as the ionization constants of four of its esters but, in order to confirm the validity of the method, we first measured the ionization constant of benzoic acid.

II. Outline of Method

If D_1 is the optical density, at a given wavelength, of a solution of a weak acid, at a pH low enough for all the acid to be present in the charged form, D_2 the density of a solution of the acid at the same stoichiometric concentration but at a pH high enough for all the acid to be converted into its fully ionized salt, and D the optical density of a solution at an intermediate pH, preferably close to the pH of the acid, then α, the degree of ionization of the acid is given by

$$D=(1-\alpha)D_1+\alpha D_2,$$

and the ionization constant is given by

$$\mathrm{pK}=\mathrm{pH}-\log\,[\alpha/(1-\alpha)]-\log\,\gamma, \quad \ldots\ldots\ldots\ldots (1)$$

where $\mathrm{pH}=-\log\,\gamma_H m_H$ and γ is the activity coefficient of the anion of the acid, that of the undissociated molecule being taken as unity in the dilute solutions

Table 2

BUFFERS USED IN THE PRESENT WORK

Ac, acetate; Cit, citrate; For, formate; Ox, oxalate; Suc, succinate

	Buffer	pH	$-\log\,\gamma$	Reference
A	(0·05M H_3Cit)	2·238	0·036	Bates, Pinching, and Smith (1950)
B	(0·01M H_3Cit)	2·624	0·024	Bates, Pinching, and Smith (1950)
C	0·01M KHOx.H_2Ox	2·156	0·057	Bower, Bates, and Smith (1953)
D	0·05M H_2Suc+0·025M NaHSuc+0·05M NaCl	3·784	0·094	Pinching and Bates (1950)
E	0·01176M HFor+0·01466M KFor+0·01269M KCl	3·795	0·067	Calc. from Harned and Embree's (1934) data
F	0·1M NaHSuc+0·1M NaCl..	4·684	0·117	Pinching and Bates (1950)
G	0·01M HAc+0·01M NaAc ..	4·718	0·044	Bates, Pinching and Smith (1950)

used. Previous experience has shown that γ can be calculated at 25 °C by a slight modification of Davies' (1938) equation

$$-\log\,\gamma=0{\cdot}5092\sqrt{I}/(1+\sqrt{I})-0{\cdot}2I, \quad \ldots\ldots\ldots (2)$$

where I is the total ionic strength.

In the present work the buffers used are those listed in Table 2. For acids of low pK, dilute solutions of hydrochloric acid of known concentration can be used to give solutions of pH between 2 and 3. All measurements were made in an air-conditioned room at 25±1 °C using a Uvispek instrument with 1-cm cells.

III. Experimental

(*a*) *Benzoic Acid*

Table 3 presents details of the experimental data. The result is in good agreement with the conductivity data of Brockman and Kilpatrick (1934), pK=4·201, and of Saxton and Meier (1934), pK=4·200, and the e.m.f. data of Jones and Parton (1952), pK=4·201, thus confirming the validity of the method and providing a useful cross-check on the buffer solutions.

Table 3

Ionization constant of benzoic acid

Wavelength: 240 mμ; concentration: $8{\cdot}2\times10^{-5}$N

Buffer	*D*	pH	α	pK
N HCl	$D_1=0{\cdot}581$			
E	0·475	3·795	0·312	4·205
F	0·309	4·684	0·800	4·199
G	0·315	4·718	0·782	4·206
0·01N NaOH	$D_2=0{\cdot}241$			

(*b*) *Esters of* p-*Aminobenzoic Acid*

We regard these as acids ionizing as

$$NH_3^+C_6H_4CO_2R \rightarrow H^+ + NH_2C_6H_4CO_2R,$$

so that modifying equation (**1**) it becomes

$$pK = pH - \log[\alpha/(1-\alpha)] + \log\gamma.$$

The experimental data are given in Table 4. The two sets for the ethyl ester show the agreement between measurements made in different laboratories, using different instruments and chemicals, and the two sets for the propyl ester illustrate the agreement at different wavelengths, one at the peak of the absorption (386 mμ) and one slightly off-peak. Previous data for the ethyl ester vary from 2·366 by Klotz and Gruen (1945) and 2·46 by Johnston (1906) to 3·13 (at 16 °C) by Kolthoff (1925); Klotz and Gruen give 2·404 for the methyl ester.

(*c*) p-*Aminobenzoic Acid*

This acid presents a more difficult problem because it is essentially a dibasic acid with ionization constants separated by about 2·4 pK units. If, for brevity, we put Z^+=the $NH_3^+C_6H_4CO_2H$ cation, Z^-=the $NH_2C_6H_4CO_2^-$ anion, $Z^\pm$=the $NH_3^+C_6H_4CO_2^-$ zwitterion, Z°=the $NH_2C_6H_4CO_2H$ neutral molecule, and $Z=Z^\pm+Z^\circ$, then ionization can occur as follows:

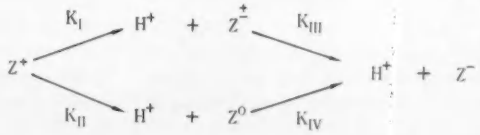

TABLE 4

IONIZATION CONSTANTS OF ESTERS OF *p*-AMINOBENZOIC ACID

Medium	Methyl Ester			Ethyl Ester			Ethyl Ester			*n*-Propyl Ester			*n*-Propyl Ester			*n*-Butyl Ester		
	Conc. $=3\cdot3\times10^{-5}$N* $\lambda=284$ mμ†			Conc. $=4\cdot3\times10^{-5}$N $\lambda=285$ mμ			Conc. $=3\times10^{-5}$N $\lambda=285$ mμ			Conc. $=5\cdot6\times10^{-5}$N $\lambda=286$ mμ			Conc. $=5\cdot6\times10^{-5}$N $\lambda=300$ mμ			Conc. $=4\times10^{-5}$N $\lambda=286$ mμ		
	D	α	pK	*D*	α	pK	*D*	α	pK	*D*	α	pK	*D*	α	pK	*D*	α	pK
N HCl ..	$D_1=0\cdot022$			$D_1=0\cdot008$			$D_1=0\cdot007$			$D_1=0\cdot010$			$D_1=0\cdot004$			$D_1=0\cdot005$		
0·01N HCl ..	—	—	—	0·180	0·236	2·512	—	—	—	—	—	—	—	—	—	—	—	—
0·008N HCl	—	—	—	0·213	0·281	2·506	—	—	—	—	—	—	—	—	—	—	—	—
Buffer *A* ..	—	—	—	0·255	0·339	2·492	—	—	—	—	—	—	—	—	—	—	—	—
0·005N HCl	0·240	0·409	2·463	0·289	0·386	2·506	0·205	0·391	2·496	0·387	0·395	2·490	0·298	0·397	2·486	0·269	0·406	$2\cdot468_5$
0·004N HCl	0·269	0·463	2·464	0·331	0·443	2·500	0·233	0·446	2·495	0·440	0·450	2·488	0·339	0·452	2·485	0·303	0·458	2·473
0·003N HCl	0·308	0·537	2·462	—	—	—	0·268	0·515	2·499	0·508	0·521	2·490	0·392	0·524	2·486	0·350	0·531	2·473
0·0025N HCl	—	—	—	0·411	0·553	2·513	—	—	—	—	—	—	—	—	—	—	—	—
Buffer *B* ..	—	—	—	0·418	0·563	2·491	—	—	—	—	—	—	—	—	—	—	—	—
0·002N HCl	0·358	0·630	2·470	0·456	0·615	2·500	0·319	0·615	2·497	0·605	0·623	2·485	0·465	0·622	2·487	0·414	0·629	2·473
Phosphate pH=7 ..	$D_2=0\cdot555$			$D_2=0\cdot737$			$D_2=0\cdot514$			$D_2=0\cdot965$			$D_2=0\cdot745$			$D_2=0\cdot655$		
Average ..	$pK=2\cdot46_5$			$pK=2\cdot50_3$			$pK=2\cdot49_7$			$pK=2\cdot48_8$			$pK=2\cdot48_6$			$pK=2\cdot47_2$		

* Ester concentration.

† λ = wavelength.

whereas the observed ionization constants are:

$$K_1=\frac{\gamma_{H^+}[H^+][Z]}{\gamma_{Z^+}[Z^+]}, \quad \text{and} \quad K_2=\frac{\gamma_{H^+}\gamma_{Z^-}[H^+][Z^-]}{[Z]},$$

the square brackets denoting concentrations.

These ionization constants are related by

$$K_1=K_I+K_{II},$$
$$1/K_2=1/K_{III}+1/K_{IV}.$$

It can be shown that, if

$$m=[Z^+]+[Z]+[Z^-],$$

then

$$[Z^+]=m[H^+]^2\zeta,$$
$$[Z]=m[H^+]K_1\zeta,$$
$$[Z^-]=mK_1K_2\zeta/\gamma_{H^+}\gamma_{Z^-},$$

where

$$\zeta^{-1}=[H^+]^2+[H^+]K_1+K_1K_2/\gamma_{H^+}\gamma_{Z^-},$$

and as the observed optical density is related to the optical densities of the Z^+, Z, and Z^- species by the following equation

$$Dm=D_1[Z^+]+D_2[Z]+D_3[Z^-],$$

then

$$[H^+]^2(D-D_1)+K_1[H^+]D+K_1K_2(D-D_3)/\gamma_{H^+}\gamma_{Z^-}=[H^+]K_1D_2, \quad .. \quad (3)$$

$pH=-\log \gamma_{H^+}[H^+]$, γ_{Z^-} can be calculated by equation (2), and D_1, D_3 are obtained from measurements in very acid and alkaline media respectively, but there is no direct way of measuring D_2. Therefore, equation (3) has three

TABLE 5

IONIZATION CONSTANTS OF *p*-AMINOBENZOIC ACID

Wavelength: 300 mμ; concentration: $7\cdot3\times10^{-5}$N; D_1 (in N HCl) = 0·003; D_3 (in 0·01N NaOH) = 0·195

Medium	D	Medium	D
0·01N HCl ..	0·225	Buffer *C*	0·259
Buffer *D*	0·736	Buffer *E*	0·742
Buffer *F*	0·533	Buffer *G* ..	0·547
$pK_1=2\cdot44_0$		$pK_1=2\cdot45_2$	
$pK_2=4\cdot85_2$		$pK_2=4\cdot85_0$	
Average $pK_1=2\cdot45$		$pK_2=4\cdot85$	

unknowns, namely, K_1, K_1K_2, and K_1D_2, so that measurements of the optical density in solutions of three different pH values suffice to determine K_1 and K_2. However, six solutions were used in order to get independent checks of K_1 and K_2. The experimental data are given in Table 5.

IV. Discussion

The ionization constants of the ethyl, propyl, and butyl esters of *p*-aminobenzoic acid form a gradated series, the pK values differing by 0·013 and 0·015 and it could be postulated, by extrapolation, that pK_{II} for *p*-aminobenzoic acid itself should be 2·528. On the other hand, the methyl ester does not fall in this series and its pK, 2·465, could be taken as pK_{II} for *p*-aminobenzoic acid. Using these extreme values, we get the following data:

pK_I	3·21	3·82
pK_{II}	2·528	2·465
pK_{III}	4·09	3·48
pK_{IV}	4·77	4·83
pK_z	0·68	1·35
Zwitterion (%)	17	4

where

$$K_z=[Z^{\pm}]/[Z^{\circ}].$$

The second assumption leads to the anomalous result, $pK_I > pK_{III}$, so that the first is more probable. It should be noted, however, that an experimental error in pK_I of 0·010 would lead to $pK_z=0·63$ and zwitterion=19 per cent. An entirely different approach by Willi and Meier (1956), using Hammett's rule, gives zwitterion=10 per cent. We hope to furnish further data on this method in a subsequent paper.

V. Conclusions

(i) The following ionization constants have been determined:

Benzoic acid	$pK=4·20_3$
p-Aminobenzoic acid:	
Methyl ester	$pK=2·46_5$
Ethyl ester	$pK=2·50_8$
n-Propyl ester	$pK=2·48_7$
n-Butyl ester	$pK=2·47_2$
p-Aminobenzoic acid	$pK_1=2·45$,
	$pK_2=4·85$

(ii) *p*-Aminobenzoic acid forms zwitterions to a small extent only, namely, of the order of 17 per cent.

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THE EFFECT OF FREE VALENCIES ON THE CHEMICAL PROPERTIES OF AZACARBONS AND CARBONS

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Summary

Carbons of high nitrogen content were prepared by charring urea-formaldehyde condensation products, and their adsorption properties studied after activation at various temperatures. The products were found to have only low basicity, which, it is suggested, is partly due to the presence of positive holes formed in the π-band during devolatilization (removal of hydrogen atoms and substituents) of the carbons. The quinone character of the carbons and their behaviour in adsorption and rubber reinforcement are also discussed in the light of this theory. It is suggested that the stabilization of devolatilized carbons, their facile adsorption of oxygen and resistance to hydrogenation, is explained by the presence in the σ-band of unshared electron pairs resulting from the formation of the π-band holes.

I. Introduction

On heating organic materials to progressively higher temperatures (pyrolysis), planar polynuclear aromatic systems of increasing size are formed. On pyrolysis of nitrogen-containing compounds, nitrogen may be incorporated into the aromatic structures. The products of pyrolysis are called carbons whether or no they contain atoms other than carbon. These carbons are intermediates between the well-defined aromatic compounds and graphites. Not all the properties of carbons can be accounted for by the extrapolation of properties of aromatic compounds. From physical measurements Mrozowski (1952) and his school suggested that on heat treatment of carbons above approximately 700 °C, devolatilization occurs at the edges of the lamellae formed by the polynuclear aromatic systems, and unshared electron pairs appear in the σ-band and positive holes in the π-band. As an extension of the work on azacarbons some of the chemical implications of this concept on carbons in general are examined in the present paper.

II. Review of the Literature

(a) *The Change of Properties of Polynuclear Aromatic Compounds with the Increase of Molecular Weight*

It can be seen from the examination of the resonance structures of aromatic compounds, that the bond order (and polarizability) is higher for bonds on the exterior of the molecule than for those contained internally. Some C—C bonds on the edges have double-bond character approximating that of ethylene

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(e.g. pyrene has two bonds of 83 per cent. double-bond character), whilst in the interior bonds are approximately 33 per cent. double bond.

An increase in the double-bond character of certain C—C linkages corresponds to the localization of π-electrons and this localization accounts for the change of properties with the increase of size, change of shape or substituents of fused benzene ring systems. With increasing double-bond character the colour deepens, the compounds have an increasing tendency to undergo addition rather than substitution reactions, the hydroxy forms of phenols become less stable relative to the keto form (Clar 1952 ; Badger 1954).

A quinone structure is also strongly influenced by the electronic properties of the molecule. Any group that is electron-donating increases the stability of the quinone relative to the quinol (Ferguson 1952) by stabilizing a negative charge on the oxygen atom. The redox potential is therefore reduced and the typical quinone reactions are absent. For example, the redox potential of *p*-benzoquinone is 0·711 V whilst that of anthraquinone is 0·155 V (Fieser and Conant 1924). The former gives all the quinone reactions whilst the latter shows nearly no quinone character at all (Evans and de Heer 1950).

The same effect may be deduced from absorption spectra and dipole moments of polynuclear compounds. Nagakura and Kuboyama (1954) showed that the electron migration from carbon atoms in the ring to oxygen atoms increases with addition of benzene rings. Handa (1955) determined the basicities of polynuclear hydrocarbons and quinones in concentrated sulphuric acid by absorption spectra measurements. He found an increase of basicity with increasing molecular weight ; the increase was specially marked in molecules with localized π-electrons.

(*b*) *The Formation of Carbons*

Several authors have described the gradual growth of a condensed benzene ring system with increasing temperature during heat treatment. X-ray diffraction measurements suggest that the diameter of a sugar char lamella reaches about 18 Å at 1000 °C (Franklin 1951). The lamellae of carbon blacks were found to be in the order of 23–28 Å (Biscoe and Warren 1942 ; Schaeffer, Smith, and Polley 1953). Also from X-ray investigations, Franklin (1951) concluded that in carbons heat treated around 1000 °C there are on the average four lamellae stacked one above the other, and these crystallites are cross-linked to others on their peripheries. She suggested that, in a sugar char heat treated at 1000 °C about 15 per cent. non-graphitic carbon forms the cross-linkages. In rubber grade carbon blacks the amount of non-graphitic carbon is roughly between 7 and 30 per cent. (Alexander *et al.*, unpublished X-ray analysis ; see Studebaker 1955). It might consist of aliphatic chains or, as considered more likely by Studebaker, of a network of highly cross-linked benzene rings (Gibson, Holohan, and Riley 1946).

(*c*) *Unsaturated Character of Crystallite Edges*

If during the process of pyrolysis a number of edge atoms lose their attached atoms, the latter take one of the valence electrons with them and the carbon

atoms are left with uncompleted octets. In other words, unpaired electrons are left on the carbon atoms (Mrozowski 1952). It should be possible to detect

these by paramagnetic resonance absorption measurements, and two groups have performed these measurements on carbons activated at varying temperatures (Ingram and Bennett 1954 ; Bennett, Ingram, and Tapley 1955 ; Ingram and Tapley 1955 ; Winslow *et al.* 1955). The absorption was found only in chars carbonized below 650 °C, the concentration of the unpaired electrons being 10^{18} to 10^{19} per g carbon. A similar finding was made by Etienne and Uebersfeld (1954) on coal.

On heat treatment of carbons above approximately 700 °C, the process of devolatilization increases sharply (see above). The absence of paramagnetic resonance absorption above 650 °C suggested that the unpaired electrons became paired. From the examination of the thermoelectric power, Hall coefficient, and electric conductivity of carbons, Mrozowski and his school (Mrozowski 1952 ; Kmetko 1953, 1954 ; Pinnick 1953) concluded that resonance electrons jump into the planar valence orbitals ($\pi \rightarrow \sigma$ transitions take place) and form pairs* with the single electrons there, leaving positive holes in the π-band (see below).

III. Experimental

The aim at first was to prepare a low-temperature char containing considerable amounts of nitrogen. Several reactions were tried, but of these the charring of urea-formaldehyde condensation products only gave carbons of high nitrogen content. Work was therefore concentrated on these only.

A few experiments were conducted to show how the reaction of carbon with a basic compound, 2-vinyl pyridine, influences the acid and alkali uptakes.

(*a*) *Techniques*

(i) *Preparation of Urea-Formaldehyde Carbons.*—An aqueous solution of formaldehyde, urea, and ammonia in the mole proportion of 3 : 1 : 0·03 was heated to boiling, let stand overnight, again boiled, and ammonia (0·3 mole) was added. A precipitate (probably iron impurities) was eliminated by filtration. The mother liquor was evaporated and the resultant gel charred in an Erlenmeyer flask under a reflux condenser (char *A*, relative absence of air), or in an open silica dish (char *B*). The chars were heated for 4 hr in tube furnaces in the stream of gases indicated (see below), let cool in the same gas (carbons activated in oxygen at 800 and 600 °C were cooled under nitrogen to avoid introduction of phenolic hydroxyls), and extracted with water in Soxhlets. The carbons were dried at 80 °C/2 mm for 24 hr. Char *A* was heated to 1000 °C for 2 hr in nitrogen or hydrogen (hydrogen was used instead of ammonia as the latter would dissociate at 1000 °C anyway).

* These unshared electron pairs are not carbanions. The latter have their octet completed.

(ii) *Adsorption Measurements.*—The acid and alkali uptakes were performed by shaking for 48 hr samples (0·5 g) of carbons with 30 c.c. of a solution containing 20 c.c. of 0·02N hydrochloric acid or sodium hydroxide respectively. After filtration a sample of the mother liquor (excess acid was added for alkali uptakes) was titrated to bromthymol blue indicator. The adsorption curves were obtained by shaking samples of carbons with 30 c.c. solutions containing increasing amounts of hydrochloric acid, filtering, and determining the acid in excess by titration with sodium hydroxide. The pH was obtained by taking the negative logarithms of the concentration of the acid.* For iodine uptake measurements, the samples (0·5 g) were shaken for 48 hr with an aqueous iodine solution (30 c.c. ; 0·0667N), filtered, and the iodine determined in the mother liquor.

(iii) *Examination for Nitrile Groups.*—Samples (1 g) of carbons were refluxed with potassium hydroxide (3 g) in glycerol (30 c.c.). A slow stream of nitrogen (saturated with water) was passed through the boiling suspension and led into standard acid.

(iv) *Quaternization Experiments.*—Samples (3 g) of carbons were shaken with methyl iodide (1 c.c.) in acetone (30 c.c.) for 48 hr. The suspensions were evaporated to dryness *in vacuo* at room temperature. Samples (1 g) of these carbons were washed with ethanol for 2 weeks. Aqueous ammonium sulphate (50 c.c. ; 0·05N) was then passed through for 24 hr to displace the iodide ions.

(v) *Reaction with 2-Vinyl Pyridine (2VP).*—A commercial carbon, " Black Pearl " (a pelletized pigment black ; see Garten and Weiss 1955), was heated in a tube furnace for 2 hr in oxygen-free nitrogen at 800 °C and let cool in the same gas. In one experiment, air was admitted when the temperature fell to 400 °C. When the temperature fell to 90 °C, nitrogen saturated with the monomer was passed through the furnace. In other experiments, carbon (10 g) was reacted with 10 c.c. of the redistilled monomer in benzol or water. In the former the catalyst used was benzoyl peroxide, in the latter potassium persulphate. The experiments were conducted under oxygen-free nitrogen. After the reaction the samples were extracted with benzene (the sample treated in water was extracted with water too).

(*b*) *Results*

(i) *Adsorption Measurements.*—After charring, the product from the urea-formaldehyde condensation contained 30 per cent. nitrogen.† Adsorption by these chars was measured after heat treatment at 400, 600, and 800 °C in

* The pH values determined by pH-meter were in some cases slightly higher, in others lower than the values obtained by titration.

† By other methods the following percentages of nitrogen could be incorporated into chars : (i) amination of chlorinated carbons at pressures up to 90 atm, 1·7 ; (ii) reaction of low-temperature chars with aliphatic aldehydes and ammonia or urea, 2·8 ; (iii) reduction and charring of *m*-dinotrobenzene, 8·0 ; (iv) charring of aniline sulphate, of aniline sulphate and glycerine, or hexamine, glycerine, and sulphuric acid, 4·9 ; (v) saw-dust charred with ammonium chloride, 8·2 ; (vi) saw-dust charred in ammonia at 400 °C, 1·3 ; (vii) saw-dust charred in ammonia at 800 °C, 1·1.

nitrogen, oxygen, or ammonia. Nitrogen as an inert gas was used to determine how heat alone modifies the carbons. Oxygen is known to give active carbons. It was hoped that ammonia might increase the basic characteristics of the carbons. Below 800 °C the nitrogen content was not influenced by the gas used for activation. It was 25–26 per cent. at 400 °C, about 22 per cent. at 600 °C, and 11–13 per cent. at 800 °C.

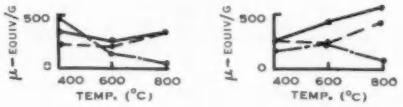


Fig. 1.—Effect of activation temperature on the acid uptake. *Left:* Char *A*. *Right:* Char *B*. —— Ammonia. — — Oxygen. —·— Nitrogen.

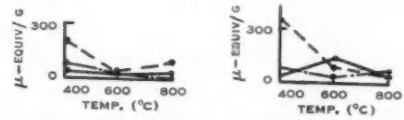


Fig. 2.—Effect of activation temperature on the alkali uptake. *Left:* Char *A*. *Right:* Char *B*. —— Ammonia. — — Oxygen. —·— Nitrogen.

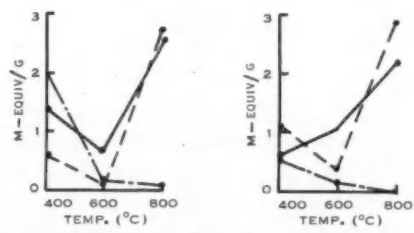


Fig. 3.—Effect of activation temperature on the iodine uptake. *Left:* Char *A*. *Right:* Char *B*. —— Ammonia. — — Oxygen. —·— Nitrogen.

From Figures 1–3, it can be seen that heating in nitrogen in nearly all cases decreased the uptakes of the carbons (there is a slight increase in the acid uptake of carbon (*B*) on heating to 600 °C. Garten and Weiss (1955) showed that the surface area of carbons increases with the temperature of the heat treatment. Assuming that a similar increase takes place in the case of azacarbons, this could outweigh the fall in adsorption due to nitrogen treatment). The alkali uptakes of the 400 °C chars were higher after oxygen than after ammonia treatment, probably due to the formation of phenolic hydroxyl groups. At 600 °C both were the same. This can be explained by the less pronounced phenolic character of these carbons (Garten and Weiss 1955). The acid uptakes of the carbons activated in oxygen or ammonia did not change considerably

with the activation temperature. Due to the increase in surface area with heat-treatment temperature (see above) the acid uptakes per unit area must have decreased considerably with the increase of the temperature of heat treatment. The iodine uptakes increased on activation in oxygen or ammonia at 800 °C.* Char *A* heated to 1000 °C for 2 hr in nitrogen or hydrogen gave acid uptakes of 20 and 70 μ-equiv/g respectively, whilst the nitrogen contents were 3·96 and 7·36 per cent. and the oxygen contents 0·67 and 3·68 per cent. respectively.

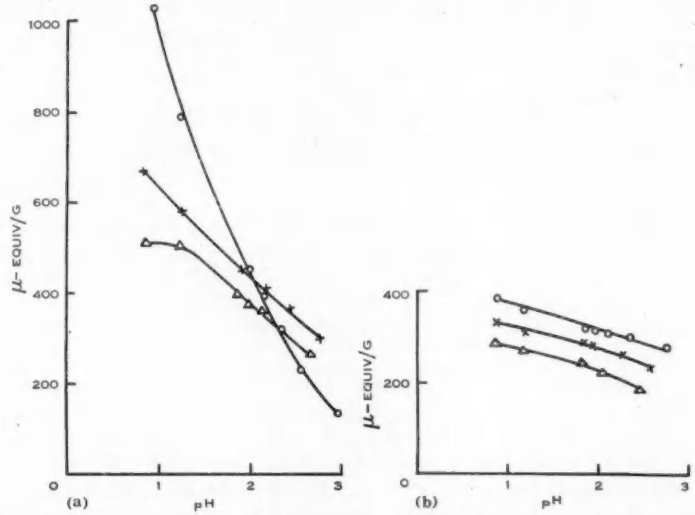


Fig. 4 (*a*).—Effect of pH on the acid uptake of nitrogen-containing char and azacarbons.
○ Untreated char *A*.
△ Char *A* heated at 800 °C in ammonia.
× Char *B* heated at 800 °C in oxygen.

Fig. 4 (*b*).—Effect of pH on the acid uptake of non-nitrogenous carbons.
○ "Black Pearl" heated at 800 °C in nitrogen.
× "Carbolac 2" heated at 800 °C in nitrogen.
△ Sugar char heated at 800 °C in oxygen.

The adsorption curves show that in the pH range examined the rate of increase of the acid uptake with the decrease of pH is much more pronounced for azacarbons (Fig. 4 (*a*)) than for carbons (Fig. 4 (*b*)).

(ii) *Alkaline Hydrolysis.*—Carbon (*A*) activated in ammonia at 600 °C liberated 38 μ-equiv of base/1 g carbon in 1 hr. After activation at 800 °C, 123 μ-equiv were liberated in 2·5 hr and further 15–15 μ-equiv in each consecutive hour.

* The activations are not easily reproducible. Adsorption measurements on different batches can give results differing by up to 30 per cent. The same was found for sugar-char carbons by Garten and Weiss (1955).

(iii) *Quaternization Experiments.*—Only with chars was it possible to liberate some iodide ions. After heat treatment (even at 400 °C) the carbons did not quaternize under the experimental conditions used.

(iv) *Reaction with 2-Vinyl Pyridine.*—The results of these experiments are given in Table 1.

TABLE 1

REACTION WITH 2-VINYL PYRIDINE

The uptakes are given in μ-equiv/g carbon

Carbon	Reaction		N (%)	2-VP on Carbon* (%)	Uptakes		
	Phase Temp. (°C)	Time (hr)			Acid	Chloride	Alkali
"Black Pearl"	Starting material (800 °C in N_2)				310		170
Sugar char† ..	Starting material (800 °C in O_2)				300	220	20
"Black Pearl"	Gas 90	72	0·24	1·8	170	100	150
"Black Pearl"‡	Gas 90	72	0·32	2·4	70	20	200
"Black Pearl"§	Benzol 80	4	0·26	1·9	230	180	Nil
Sugar char ..	Water 60	3	0·20	1·5	120	80	20
Sugar char ..	Benzol 70	1	0·98	7·3	40	10	30

* Calculated from the nitrogen content.

† Different batch from the one used for the determination of the adsorption curve.

‡ During cooling, air was admitted when the temperature fell below 400 °C.

§ Air was not excluded during this experiment.

IV. Discussion of Experimental Results

The question arises as to the form in which the nitrogen is present and why it is non-basic. It might be in side chains such as nitrile or in graphitic layers.*

(*a*) *Nitrile Groups*

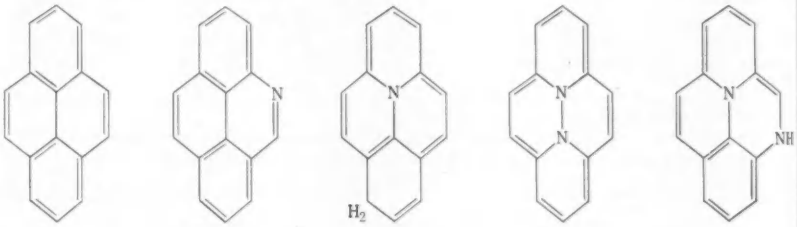
It is unlikely that nitrogen in side chains could withstand temperatures of 800 °C in any form other than nitrile groups. The slow rate of hydrolysis even at 300 °C suggested the absence of nitrile groups; the small amount of base liberated might be due to heterocyclic nitrogen decomposing into ammonia. However, the alkaline hydrolysis of aliphatic nitriles, RCN, is facilitated by electron release from R (Remick 1950); accordingly, it is possible that nitrile groups on carbons are deactivated if the carbon behaved as an electron sink. Therefore, it is not proved that nitrile groups are absent.

Analysis of the 800 °C carbon shows that the atomic ratio of C : N is about 6, that is, there should be five carbon atoms for every —CN group. It is expected that about 60 per cent. of the carbon will be used to make up the inside of the

* X-ray measurements, kindly performed by Mr. A. D. Wadsley, Division of Industrial Chemistry, showed that the urea-formaldehyde carbons are of similar graphitic structure to the sugar carbons.

graphitic layers, where it cannot be substituted (see below). Consequently, there will be one nitrile group for every two carbon atoms on the edges. In big aromatic lamellae every second edge atom is linked to three carbon atoms and therefore cannot be substituted. From this reasoning it follows that every available site should be substituted by nitrile groups, but since these carbons contain considerable amounts of hydrogen that must be accommodated as well it is unlikely that a major part of the nitrogen is present as nitrile groups.

Much of the nitrogen could be incorporated into the graphitic layers.* In aromatic compounds nitrogen can replace any of the CH groups on the edges. For example, pyrene (I) could give 10 such monoaza-derivatives (II) and in all these the resonance energy would not differ greatly from that of the parent compound. If, however, nitrogen replaces a carbon atom in the interior of the plane either a CH_2 group has to be formed on the periphery (III), or another nitrogen atom has to be introduced into the interior of the plane (IV), or an NH group introduced in certain positions on the edge of the molecule (V). The resonance energy and the entropy change both favour the formation of compounds with nitrogen on the edges compared to those with nitrogen in the interior of the planes.



(I) (II) (III) (IV) (V)

Loss of nitrogen at higher temperatures is facilitated if the nitrogen is preferentially incorporated into the edges as loss from the interior of the planes would require a rearrangement of the whole structure.

(*b*) *The Basicity of Nitrogenous Carbons*

The acid uptakes of the urea-formaldehyde carbons are far less than would correspond to the salt formation of all the nitrogen present. For example, an acid uptake of 600 μ-equiv/g carbon corresponds to 0·8 per cent. nitrogen. This suggests that either the nitrogen is in a relatively non-basic form or it is not accessible to the acid. The fact that no reaction could be detected with methyl iodide suggested that the nitrogen is non-basic.

The same conclusion can be drawn from the examination of the adsorption curves. Figure 5 shows the percentage ionization of a base with the change

* Working on coal, Heathcoat and Wheeler (1932) came to the same conclusion. They found that on chlorination a considerable portion of the nitrogen was retained in the non-volatile product. This has been taken as an indication that the nitrogen in coal is in a highly stable form and is an integral part of the ring structure.

in pH (Albert 1947). The rate of increase of ionization is much larger at hydrogen ion concentrations lower than the pK_a value than at higher ones. For example, it increases from 9 to 50 per cent. (an increase of 456 per cent.) in one pH unit on the alkaline side of the pK_a value but only from 50 to 91 per cent. (an increase of 80 per cent.) on the other side. Though conditions are much less clear-cut where the compounds contain several basic groups, the adsorption curves suggest that the nitrogenous carbons are either less basic than the non-nitrogenous carbons (the curves in Fig. 4 (*a*) show a bigger rate of increase at lower pH values than those in Fig. 4 (*b*)) or their adsorption curves show the superposition of adsorption due to two different mechanisms. At higher pH values the adsorption might be due to the usual acid adsorption on carbons (see below), whereas at

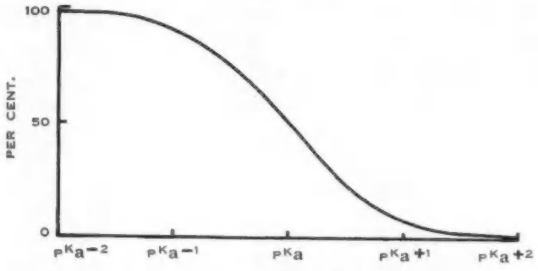


Fig. 5.—Effect of pH on the ionization of a base.

low pH values it might be due to the salt formation of weakly basic nitrogen compounds. Support for the latter explanation comes from the finding that the untreated char—containing large amounts of nitrogen—gave the biggest rate of increase in adsorption (Fig. 4 (*a*)).

The following explanations are offered for the low basicities of the carbons :

(i) Quinone groups or adsorbed oxygen may shift the negative charge from the nitrogen atoms by inductive effect. The oxygen content of an 800 °C carbon is about 7 per cent. ; a lamella of about 17 Å diameter—consisting of about 100 carbon atoms—should therefore contain approximately six oxygen atoms. On heating carbons to 1000 °C, the acid uptakes decreased compared to the 800 °C carbons, though the N : O ratio increased from 1·86 at 800 °C to 2·0 and 5·9 when the carbon was heated at 1000 °C in hydrogen or nitrogen respectively. The oxygen content is therefore not solely responsible for the lack of basicity.

(ii) All the aromatic heterocyclic compounds related to pyridine are bases with pK_a values of approximately 5·0. The introduction of a second nitrogen atom into a six-membered ring greatly reduces the basic strength, because the nitrogen is electron attracting. It attracts the lone pair of the first nitrogen thereby reducing its availability for salt formation (Badger 1954). An 800 °C carbon, containing about 13 per cent. nitrogen, would have approximately

12 nitrogen atoms per lamella of 17 Å diameter so that reduced basicity is to be expected.

(iii) The positive holes formed by the Mrozowski type electron distribution should be electron attracting.

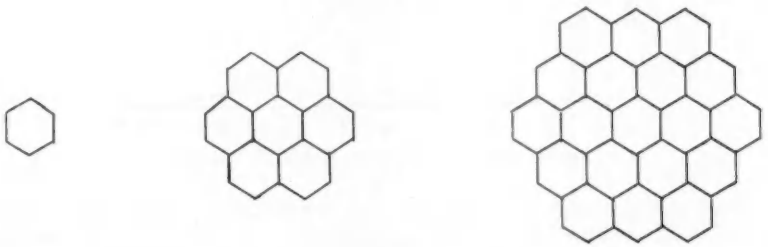
(c) *Reaction with 2-Vinyl Pyridine*

The addition of 2-vinyl pyridine reduced the original acid uptakes. In some cases the acid uptake was even less than would correspond to the salt formation of the nitrogen introduced.* This is an indication of the electron-attracting character of the carbons.

V. Theoretical Considerations Based on Results Published in the Literature

(a) *Distribution of Electrons on the Edges of Lamellae*

The number of free valencies on the edges of the lamellae may be examined by taking the proportion of edge atoms to plane atoms as the lamella grows (Table 2).† Earlier it was noted that carbon blacks have lamellae of 23–28 Å



in diameter and 7–30 per cent. of their carbon is in non-graphitic form. From these figures the minimum amount of hydrogen that the carbon must contain

Table 2

Growth of the Lamella

Diameter (Å)	2·4	7·2	12·0	16·8	21·6	26·4
Number of:						
Benzene rings ..	1	7	19	37	61	91
Inside atoms ..	Nil	6	24	54	96	150
Edge atoms ..	6	18	30	42	54	66
Edge atoms (%) ..	100	75·0	55·6	43·7	36·0	30·6

* A sample of " Black Pearl " reacted with styrene vapour after heat treatment gave an acid uptake of 190 μ-equiv/g. This showed that reaction with a vinyl compound did not eliminate the basicity of the carbon. It is therefore probable that after reaction with 2-vinyl pyridine the acid uptake is not due to salt formation of the pyridine groups only.

† It is realized that this picture is only a very rough approximation; also, in the low-temperature carbons there would be aliphatic side chains present.

if no unsaturated valencies are present can be calculated.* The number of carbon atoms requiring attached hydrogen atoms (or substituents) is the smallest under the following conditions :

(i) The percentage of non-graphitic carbon is large (assuming that all this is of the type described by Gibson, Holohan, and Riley (1946) and consisting only of carbon atoms).

(ii) The diameter of the lamella is large.

(iii) The lamella is of the regular shape shown opposite.

A channel black contains 3·6 per cent. oxygen and 0·6 per cent. hydrogen (Kirk and Othmer 1949). Supposing that only 70 per cent. of the carbon is in the graphitic form and only 30 per cent. of the latter forms the edges and half of these could be substituted, we obtain as C : H : O atomic ratio on the edges 4 : 2·7 : 1. Even this calculation shows a number of carbon atoms possessing free valencies. In reality, the number of unshared electron pairs and holes must be much larger for the following reasons :

(i) The maximum amount of non-graphitic carbon was used for the calculation and even the carbon described by Gibson, Holohan, and Riley (1946) contains other than carbon atoms (one foreign atom per three carbon atoms).

(ii) Thirty per cent. edge atoms corresponds to lamellae with nearly the maximum diameter given by X-ray analysis. Smaller lamellae have more edge atoms.

(iii) The lamella—if irregular in shape—contains a higher percentage of edge atoms.

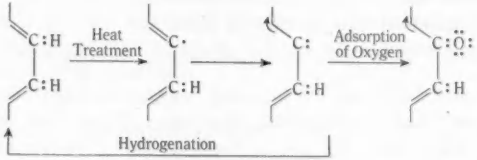
(iv) Part of the hydrogen and oxygen found by analysis is present as water and is therefore not available to saturate the valencies of the edge carbon atoms.

The number of free valencies in furnace blacks must be much higher than in channel blacks because the former contain less hydrogen and oxygen (0·4 and 0·3 per cent. respectively ; Kirk and Othmer 1949).

Considering the carbons contain a large number of atoms with unsaturated valencies, one would expect them to be readily hydrogenated. Kmetko (1954) found that this expectation was not fulfilled while the amounts of hydrogen that could be reacted by Studebaker *et al.* (1956) were relatively small. Smith *et al.* (1956) found that hydrogen treatment of carbons at 1000 °C removes oxygen complexes but C—H complexes are not formed in their place ; the hydrogen content is even reduced. In air the carbon picks up oxygen again. Accepting the Mrozowski model, the above phenomena can readily be explained. For hydrogenation, one of the electrons of the unshared electron pair has to be raised into the π-band so that the valence electron of the hydrogen can take

* Though some of the free valencies can be engaged in intercrystalline bonding, the number of these must be limited, otherwise this type of bonding would correspond to an increase in the diameter of the lamellae.

its place. This change in the energetically favoured electron distribution certainly requires energy. The adsorption of oxygen with its sextet does not require the uncoupling of the unshared electron pair. It is possible that the C—O bond thus formed will be a double bond, an electron pair of the oxygen being shared with the carbon atom, and not a single bond as shown in the diagram :



The excess of positive holes over unshared electron pairs after the adsorption of oxygen should render the carbons electron attracting.

The findings of Uebersfeld and Erb (1955) give added support to the above theory. They showed that sugar char heat treated at 650–850 °C gave paramagnetic resonance absorption (10^{20} radicals/g) when examined *in vacuo* or nitrogen. On admission of oxygen the absorption disappeared in 10–20 sec. Heated above 900 °C no resonance absorption could be detected even *in vacuo*.

It is now suggested that the explanation of all these findings is as follows :

(i) Below 650 °C, the carbons are insulators. Due to the energy gap between the π- and σ-bands, $\pi \rightarrow \sigma$ transitions take place. In this process unpaired electrons are formed in the π-band. These are localized and give paramagnetic resonance absorption.

(ii) Between 650 and 850 °C, in the absence of oxygen, conditions are similar to (i). When oxygen is present, due to its inductive effect the number of holes in the π-band increases. This decreases the electric resistance (Mrozowski and his school showed that these carbons are hole type conductors) and the single electrons can become paired.

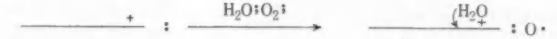
(iii) Above 900 °C, the carbons are conductors and all the electrons can become paired.

(*b*) *Adsorption*

On the planes of carbon lamellae, van der Waals type adsorption should predominate whilst on the edges—due to higher bond-order and to the Mrozowski type electron distribution—adsorption could result from the interaction of much stronger forces.

Busso *et al.* (1950) showed by quantum mechanical calculation that the edge atoms of graphite layers are reactive, whilst the atoms on the inside of the planes are relatively inert. In a review article (Busso 1950), experimental evidence is presented to support the above calculation (oxidation and adsorption studies). Adsorption studies suggest that most of the carbon surface is hydrophobic (Young *et al.* 1954) but water is adsorbed on part of the surface which is probably the hydrophilic edges (Healey, Yung-Fang, and Chessick 1955).

The presence of both unshared electron pairs and holes on the edges of the graphitic layers suggests that the adsorption of one species should be strongly influenced by a second adsorbate. Courty (1947) found that on coconut char, after vacuum treatment, the rate of adsorption of oxygen and the amount adsorbed increased with the amount of moisture present. This could now be interpreted as the water molecules supplying electrons to the π-band* and the unshared electron pairs of the σ-band and thereby becoming more readily available to be shared with the electrophilic oxygen. According to Frumkin



and his co-workers, charcoals suspended in solutions of electrolytes behave as reversible gas electrodes (Frumkin 1930) and the adsorption on them is determined by the sign and magnitude of the charge. The objections to this theory are discussed by Steenberg (1944). Nevertheless adsorption of oxygen on unshared electron pairs can be considered as imparting a positive charge to the carbon. Similarly, adsorption of hydrogen on platinized carbon might impart a negative charge to the carbon. Also, the small adsorptive capacity of degassed

carbons can be explained as adsorption taking place on unshared electron pairs and on positive holes. According to Steenberg (1944) organic molecules are more readily adsorbed on carbons than inorganic ions. Considering the higher polarizability of the former, this finding is to be expected if the Mrozowski model is accepted.

(c) *Quinone Groups*

An inductive effect produced by the holes could explain the quinone character of the carbons, which character should decrease as the size of the lamellae increases (see above). If the temperature of activation is increased, the oxygen content of carbons decreases. It is expected that the oxygen will be preferentially retained in positions of highest electron density. Thus the negative charge on the oxygen should be even more accentuated and the quinone character should be absent. In contradiction to this reasoning, three groups of workers showed the presence of quinones on carbons. Dr. W. V. Smith (personal communication; see Barton, Smallwood, and Ganzhorn 1954) described the reversible reduction of carbons by ferrous ions and oxidation by air. Garten and Weiss (1955) showed the increased alkali uptakes of the reduced carbons. Studebaker (1955) and Studebaker *et al.* (1956) performed reduction experiments and reactions with diazomethane. It is now suggested that positive holes reduce the negative charge on the oxygen atoms with a resultant increase in redox potential. The typical quinone reactions then become apparent.

* From the change of electric conductivity of Cu_2O on the adsorption of water it could be concluded that water gave off electrons to this adsorbent (Hauffe 1955).

(*d*) *Carbon Blacks*

It is known (Studebaker 1955) that carbon blacks are both oxidizing and reducing agents. This can readily be understood, if one considers that the carbons have unshared electron pairs (reducing agents) and positive holes (oxidizing agents).

It was shown that carbon blacks can act as free-radical acceptors (Garten and Sutherland 1954; Watson 1954). This finding fits in well with the Mrozowski model. By supplying sufficient energy for the uncoupling of the unshared electron pairs, single electrons are formed that could become paired with the attacking free radicals.

Zapp and Gessler (1953) showed that heat treatment of butyl-channel black mixtures leads to pronounced improvements in the physical properties of the resulting vulcanizates. The improvements could not be obtained with furnace blacks or with channel blacks after devolatilization. Furnace black after oxidation of its surface gave the same effect as channel black. These experiments showed that the carbon was effective only if oxygen was present at its surface. One effect of the oxygen might be to prevent the shift of electrons between the σ- and π-band by being adsorbed on the unshared electron pairs and thereby making the positive holes more readily available for reaction with rubber.

VI. Conclusion

Evidence brought forward in the present paper suggests that the chemical properties of carbons are strongly influenced by the Mrozowski type electron distribution.

VII. Acknowledgments

The author is indebted to Dr. W. Zimmerman for the microanalysis, to Dr. R. D. Brown, University of Melbourne, for helpful discussion on orbital theory, to Dr. W. D. Crow, Division of Industrial Chemistry, for valuable advice and criticism, and to Dr. K. L. Sutherland, Division of Industrial Chemistry, for all the advice and help given during the course of this investigation.

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KINETICS OF THE REACTION BETWEEN MANGANESE DIOXIDE AND FERROUS ION

By D. F. A. Koch*

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Summary

The kinetics of the reaction between manganese dioxide and ferrous ion in acid solution have been investigated by using the potential of the ferrous-ferric couple as a measure of the extent of reaction. The experimental conditions were such that the reaction rate was independent of ferrous, ferric, manganous ions, and acid concentrations and the agitation was sufficient to prevent bulk diffusion in the solution from being a rate-determining factor.

The reaction rate of sized samples of pyrolusite and γ-MnO_2 in ferrous sulphate solution was proportional to the surface area of the solid and was constant (i.e. " zero-order ") until 50 per cent. of the solid was consumed. γ-MnO_2 reacted about twice as rapidly as the pyrolusite. The reaction occurred most readily at certain active sites on the particles and appeared to proceed along crystal boundaries in such a manner that the active surface area was not significantly changed during the first half of the reaction. In ferrous perchlorate the reaction rate of 10 μ diameter pyrolusite was about one-hundredth of that in sulphate and the reaction appeared to occur at a more even rate over the whole surface of the particle so that the zero-order law was no longer obeyed. Activation energies of 7·4 and 5 kcal in sulphate and perchlorate respectively, for the temperature range 18 to 40 °C, suggest that the difference in rate is a result of a change in the entropy factor of the Arrhenius equation. It is suggested that this difference in rate may result from the activation, by sulphate ions, of a less reactive lower oxide of manganese which is formed on the surface.

I. Introduction

The oxidation of ferrous ion in acid solution by solid manganese dioxide is of importance in analytical chemistry and also as a purification step in the hydrometallurgy of zinc and copper. Iron, an undesirable contaminant in zinc and copper electrolytes, is oxidized to the ferric state and subsequently removed by precipitation of the hydrous ferric oxide. Despite its importance, no reference to the kinetics of the reaction could be found in the literature. The net reaction may be represented as follows :

$$MnO_2 + 2Fe^{2+} + 4H^+ \rightarrow Mn^{2+} + 2Fe^{3+} + 2H_2O.$$

In a heterogeneous reaction of this type the rate could depend on the structure and surface area of the solid, the composition of the solution, and the temperature.

Manganese dioxide can exist in several different crystalline forms and the effect of composition, surface area, and crystal structure on the reactivity of

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the oxide has been reviewed by Wadsley and Walkley (1951). The reactivity has been measured by the electrode potential of the couple

$$MnO_2 + 4H^+ + 2e \rightarrow Mn^{2+} + 2H_2O$$

(Wadsley and Walkley 1949 ; Maxwell and Thirsk 1955), the rate of catalytic decomposition of hydrogen peroxide (Parker, Cohen, and Smith 1948) and the rate of oxidation of oxalic acid (Skwortzow 1935 ; D'Agostino 1938 ; Wadsley and Walkley 1952). Although the method of preparation of any one structural form of manganese dioxide affects its reactivity the three reactions described above all show that γ-MnO_2, produced by electrolysis, is more reactive than pyrolusite (β-MnO_2), formed by pyrolysis of manganous nitrate. Skwortzow (1935) found that the reduction of colloidal manganese dioxide by oxalic acid obeyed a rate equation which suggested that reaction occurred evenly over the whole surface of the manganese dioxide and was retarded by the products. The reaction rates for oxalic acid oxidation (Wadsley and Walkley 1952) and hydrogen peroxide decomposition (Parker, Cohen, and Smith 1948) were both proportional to the surface area of the solid for any one variety of manganese dioxide.

Although the influence of manganese dioxide structure has received considerable attention the effect of solution composition and particularly the effect of anions in solution has been relatively neglected. Consequently, most of the work described here has been carried out on sized samples of a synthetic pyrolusite with a well-defined crystal structure while the composition of the solution and temperature have been varied.

This work follows the investigation on the effect of particle-size distribution on the shape of the rate curve for the reaction between ferrous sulphate and manganese dioxide (Koch and Walkley 1956) and is an attempt to obtain further information on the mechanism of this reaction.

II. Experimental

Two structural forms of manganese dioxide have been used in the present work. An oxide with the pyrolusite structure was prepared from electrolytic manganese metal by dissolving it in " Analar " nitric acid and decomposing the nitrate at 200 °C in a silica dish. The oxide was then heated in a current of oxygen for 4 hr at 200–300 °C. The product showed the pyrolusite structure on X-ray examination (Wadsley and Walkley 1951) and chemical analysis gave an Mn : O ratio of 1 : 2·00. About 1 per cent. silica was found in the material as a result of attack on the silica ware used for its preparation. When sized in a Haultain infrasizer the silica was concentrated in the fractions with a diameter less than 40 μ. The reaction rates which were subsequently determined showed no effect which could be attributed to the varying amounts of silica in the different fractions.

The other manganese dioxide was prepared by the anodic oxidation of an aqueous solution of manganous sulphate. The oxidation occurred on a graphite anode and the resulting product contained about 1 per cent. carbon. This manganese dioxide had the γ-MnO_2 structure and was more reactive to oxalic

acid than the pyrolusite (Wadsley and Walkley 1952). Chemical analysis gave the Mn : O ratio 1 : 1·95.

Both unsized and sized samples of manganese dioxide have been used in this work. The particle-size distribution of the unsized pyrolusite is as follows : +150 μ diameter, nil ; 125±20 μ, 2·5 ; 89±15 μ, 18·7 ; 64±10 μ, 21·2 ; 50±4 μ, 10·6 ; 39±7 μ, 15·3 ; 28±5 μ, 7·8 ; 20±3 μ, 4·0 ; 14±2 μ, 2·7 ; 10±2 μ, 2·0 ; −8 μ, 15·2 per cent.

The oxidation of ferrous ion was examined in sulphate and perchlorate media. The ferrous sulphate solutions were prepared from " Analar " $FeSO_4.7H_2O$ and sulphuric acid to give a solution containing 0·074M ferrous sulphate. Ferrous perchlorate solutions of the same concentration were prepared by dissolving electrolytically produced metallic iron in " Analar " perchloric acid.

The oxidation was carried out in a 250 ml three-necked, round-bottom flask, immersed in a thermostated water-bath controlled to ±0·2 °C by a Sunvic thermoregulator. In order to eliminate bulk diffusion in the liquid as a rate-determining step the contents of the flask were agitated by a motor-driven glass stirrer at a speed well above the range in which the reaction rate depended on the rate of stirring.

The progress of the reaction was measured by the potential of the ferrous-ferric couple at a platinum wire dipping into the solution. A saturated calomel electrode was used as the standard and this was connected to the solution by a potassium chloride, agar bridge. Tests showed that the amount of chloride ion which diffused into the solution from this bridge during an experiment had no measurable effect on the rate in sulphate solutions. In perchlorate, however, there was a marked increase in rate due to chloride ion and an acid solution of ferrous perchlorate was used as a bridge. The potentials were measured to ±1 mV on a valve potentiometer.

A sample containing 0·100 g MnO_2 was added to 100 ml of the ferrous solution in the reaction vessel and the rate was followed by observing the changes in potential. The quantities of reactants used ensured that the ferrous ion concentration decreased from 0·074 to 0·051M during the reaction. Tests showed that the reaction rate was independent of ferrous concentration down to at least 0·03M, so that the experimental concentrations served the twofold purpose of eliminating the effect of ferrous concentration on the reaction rate and of maintaining a high ferrous to ferric ratio so that the potential was sensitive to small changes in the ferrous concentration. The potential change during reaction was about 120 mV.

The weight of manganese dioxide reacted, at any given time, was determined by comparing the measured potential with a calibration curve. Calibration curves for each solution were made immediately after the rate experiment to ensure that the platinum electrode was in a similar condition and also that the slow air oxidation of the ferrous solution was not significant. Weighed amounts of manganese dioxide (from 0·01 to 0·10 g) were added to 100 ml of

the initial ferrous solution and the potential measured after each addition of the oxide had completely reacted. Typical calibration curves for a series of temperatures in 0·2N sulphuric acid are shown in Figure 1. The potentials in 0·2N perchloric acid were about 0·07 V higher.

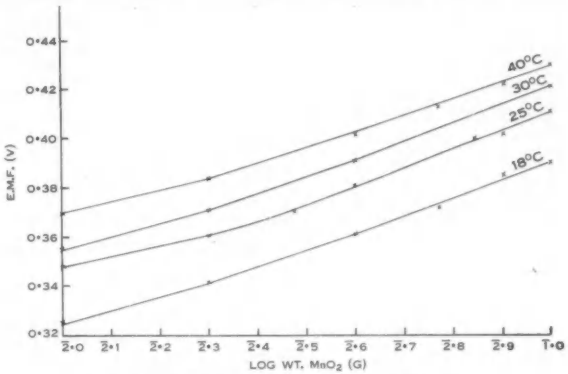

Fig. 1.—Calibration curves in 0·2N H_2SO_4.

III. Results

The rate curves (Figs. 3, 4, and 6) were obtained by plotting the weight of remaining manganese dioxide against time. The initial slopes of these curves in sulphate solution (Figs. 3 and 4) are linear and will be called the " zero-order " rates. γ-MnO_2 was used for one set of experiments only (Fig. 4) and unless otherwise stated manganese dioxide refers to synthetic pyrolusite.

(i) *Effect of Ferrous Concentration.*—When the initial ferrous sulphate concentration was reduced from 0·074 to 0·054M no difference in the rate of reaction could be detected. In this work, therefore, the rate is independent of the changes in ferrous concentration during reaction. However, in a test in which complete oxidation of ferrous sulphate was obtained with a slight excess of manganese dioxide it was found that when the ferrous concentration dropped below 0·01M the reaction was first order with respect to ferrous sulphate.

(ii) *Effect of Ferric Concentration.*—The initial ferric ion concentration was made up to 0·02M in both sulphate and perchlorate solutions containing 0·2N acid. No difference in the rate could be detected between these experiments and those without added ferric ion.

(iii) *Effect of Manganous Concentration.*—Manganous sulphate had no effect on the rate in concentrations up to 0·01M, the concentration produced during reaction. When the initial manganous concentration was raised to 0·2M, however, there was a slight retardation in the reaction rate and the effect was comparable to that of cupric ion which will be described subsequently.

(iv) *Effect of Acid Concentration.*—The reaction rates were measured in solutions ranging from 0·1 to 1·0N sulphuric acid and 0·2 to 1·0N perchloric

acid. These large changes in acid concentration did not change the rate. The independence of the reaction rate to the large changes in viscosity and ionic strength involved in these changes in acid concentration confirms the absence of bulk diffusion as a rate-determining step. In an aqueous ferrous sulphate solution to which no acid was added the reaction apparently ceased after 30 per

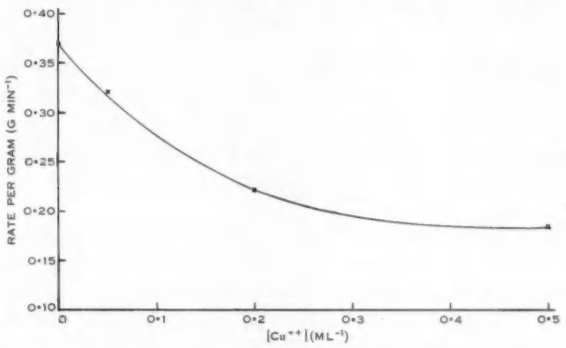

Fig. 2.—Effect of Cu^{2+} on reaction rate. Temperature 25 °C; 0·2N H_2SO_4.

cent. only of the pyrolusite had reacted. The pH of the resulting solution was 3·8 and it is probable that hydrous ferric oxide forms a protective film under these conditions.

(v) *Effect of Cupric Concentration.*—The influence of a large concentration of foreign cations on the reaction rate was a point of interest and the rates

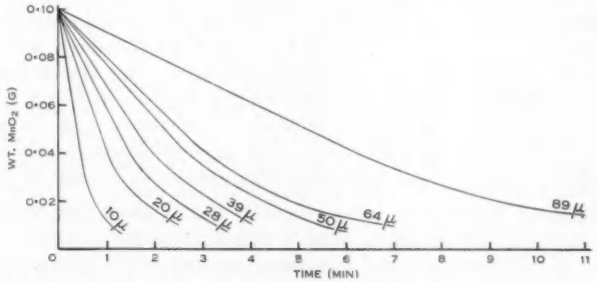

Fig. 3.—Reaction of sized pyrolusite (mean dia. shown) with ferrous sulphate. Temperature 25 °C; 0·2N H_2SO_4.

were measured in sulphate solutions containing varying amounts of cupric sulphate. The particle size of the pyrolusite was 20 μ and the rate is plotted against copper concentration in Figure 2.

(vi) *Rates in Sulphate and Perchlorate Solutions.*—The reaction rate in perchlorate was much less than that in sulphate and the shape of the rate curves also differed (Figs. 3 and 6).

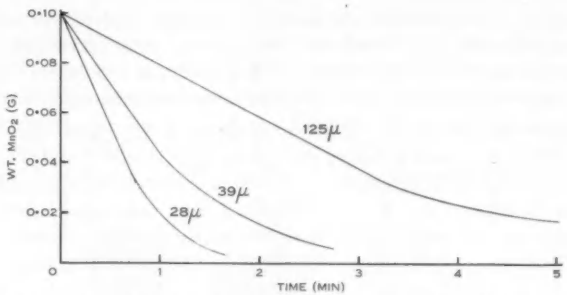

Fig. 4.—Reaction of sized γ-MnO_2 (mean dia. shown) with ferrous sulphate. Temperature 25 °C; 0·2N H_2SO_4.

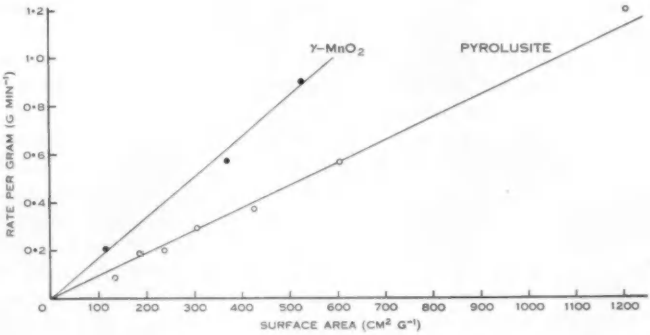

Fig. 5.—Effect of surface area on reaction rate. Temperature 25 °C; 0·2N H_2SO_4.

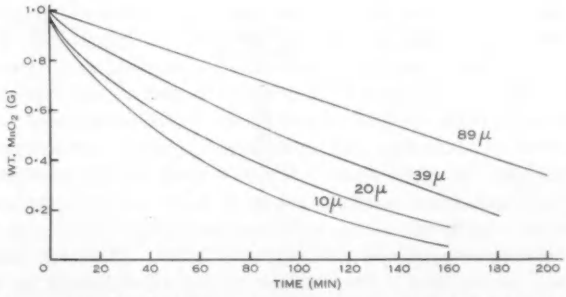

Fig. 6.—Reaction of sized pyrolusite (mean diam. shown) with ferrous perchlorate. Temperature 25 °C; 0·2N $HClO_4$.

(vii) *Effect of Temperature.*—The reaction rates of pyrolusite in both sulphate and perchlorate increased with temperature and the results gave a linear curve when the logarithm of the rate was plotted against the reciprocal of the absolute temperature in the range 18 to 40 °C. The apparent activation energies were 7·4 and 5 kcal mole^{-1} for sulphate and perchlorate solutions respectively.

(viii) *Effect of Manganese Dioxide Structure.*—The rate curves for sized fractions of pyrolusite and γ-MnO_2 are shown in Figures 3 and 4 respectively. The zero-order rate constants from these curves are plotted against the initial surface area in Figure 5. The surface area was calculated from the mean particle radius on the assumption that the particles were spheres. The same result would be obtained for cubes if half the edge length is substituted for the radius.

IV. Discussion

The reaction rate was independent of the concentrations of ferrous, ferric, and manganous ions in the ranges 0·07 to 0·05M, 0 to 0·02M, and 0 to 0·01M respectively. This suggests that the rate-determining step is neither adsorption nor desorption of these ions but one which occurs at the oxide surface independently of the solution composition of these ions. In a test in which the ferrous had been reduced to 0·01M, however, the rate was first order with respect to this component, showing that the surface concentration of ferrous ion can be an important factor. If the area available for ferrous adsorption is only a fraction of the total surface area, and this adsorption area is saturated under the experimental conditions, the rate-determining step may be the migration of an adsorbed ferrous ion, over the surface, to a reactive site where it is oxidized. The reaction rate would then be independent of the concentrations of ferrous, ferric, and manganous ions. The retarding effect of cupric sulphate (Fig. 2) may be attributed to either a blocking of the surface diffusion or deactivation of the active centres by an adsorbed foreign cation. The adsorption of cupric, nickel, and cobalt ions on manganese dioxide surfaces has been reported by Geloso and Levy (1928) and Eristavi (1948).

Evidence for active sites on pyrolusite will now be discussed. The rates of reaction of sized fractions of pyrolusite and γ-MnO_2, though dependent on the initial particle size, remain constant until about 50 per cent. of the solid is consumed (see Figs. 3 and 4). If the particles are considered as spheres or cubes of continuously decreasing radii or edge lengths, this corresponds to a surface area decrease of about 50 per cent. The zero-order kinetics are not the result of the solid being present as flat plates, which would show a very small decrease in surface area, since microscopic examination of the pyrolusite (Fig. 7 (*a*)) showed that the three axes of the particle were approximately equal.

Ferric and manganous ions, the products of the reaction, do not show any acceleration or retardation of the reaction rate which may compensate for a decrease or increase in surface area during reaction. However, the zero-order reaction could be produced if the reaction rate was determined by oxidation of ferrous ion at " active " sites on the manganese dioxide surface and if the number of these sites remained constant for the first 50 per cent. of the reaction. Photo-

micrographs of pyrolusite taken before and after partial reaction (Figs. 7 (*a*), 7 (*b*)) show that the initially smooth surface of the particles (Fig. 7 (*a*)) is appreciably pitted after 50 per cent. has reacted (Fig. 7 (*b*)) and after 70 per cent. the pitting is so deep that the particles begin to break up into fragments which vary greatly in size. The zero-order rate curves for sized samples change to first order curves when the solid is unsized and a general procedure has been described (Koch and Walkley 1956), showing how the rate curve for an unsized sample may be derived from those of sized samples, provided the size distribution of the

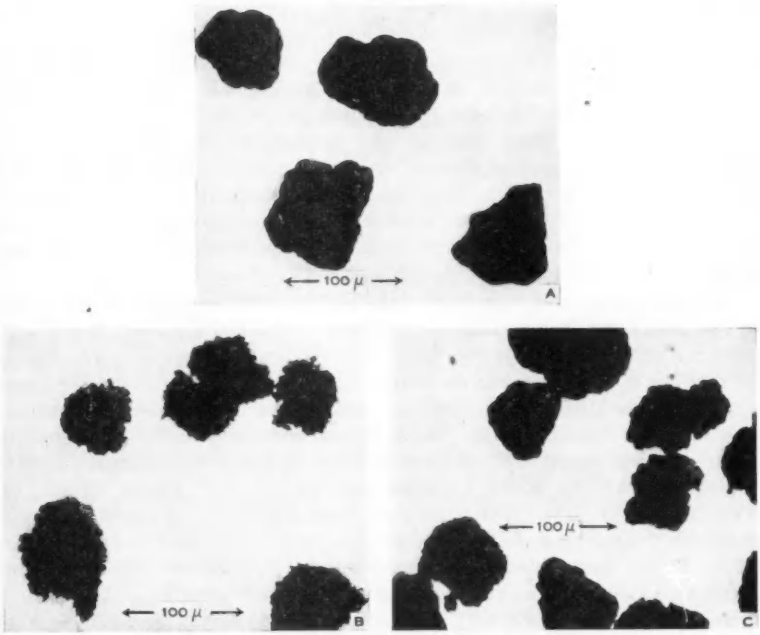

Fig. 7.—*A*, pyrolusite before reaction ; *B*, pyrolusite 50 per cent. in $FeSO_4$; *C*, pyrolusite 50 per cent. in $Fe(ClO_4)_2$.

unsized material is known. The rate curves in Figures 3 and 4 change from zero to first order after 50 per cent. of the solid has reacted and this is the region where the particles break up into fragments of varying size.

The pyrolusite particles are not single crystals and it is probable that the active sites are crystal boundaries along which the reaction may proceed more rapidly than on the remaining surface. If the small crystals are randomly oriented in the pyrolusite particle, reaction of this type could produce the pitting shown in Figure 7 (*b*).

The initial surface areas per gram of pyrolusite and γ-MnO_2 have been calculated for several sized fractions on the assumption that the particles are

cubes or spheres. If the edge length of the cube and the diameter of the particle are used for this simple geometrical calculation, both cubes and spheres have the same area for a given weight of material. This geometric area may not be the true surface area but the linear relationship obtained between reaction rate and the calculated area (Fig. 5) suggests that it is at least proportional to the true area. Figure 5 also shows that for a given particle size the γ-MnO_2 reacts about twice as rapidly as the pyrolusite. It is not possible to state whether the higher rate is caused by a twofold increase in surface area or by a difference in lattice structure since the particles of γ-MnO_2 were more irregular in shape than the pyrolusite.

The solution variable which has the greatest effect on the reaction rate is the type of anion, and the reaction of pyrolusite in sulphate solution (Fig. 3) is much more rapid than in perchlorate (Fig. 6). Although ferric sulphate complexes have been identified (Lister and Rivington 1955; Whiteker and Davidson 1955) and may increase the desorption rate of ferric ion, the independence of the reaction rate to ferric concentration shows that this is not the origin of the differences observed. Furthermore, the electron exchange between ferric and ferrous which is sensitive to the anion present is also a very unlikely rate-determining step since this is very dependent on acid concentration (Silverman and Dodson 1952).

Trivalent manganese is a likely intermediate in the reduction of the dioxide and the possibility that a manganic sulphate complex (Purdy and Hume 1955) caused the differences in reaction rate, was considered. Pyrolusite was digested in a solution of 0·1M manganous sulphate and 0·2N sulphuric acid for 15 min at room temperature. The solution was filtered and tested for oxidizing properties with diphenylamine. No manganic ion could be detected until the acid strength was raised to 5N and it is therefore unlikely that manganic sulphate is present in solution during reaction with ferrous ion. Parker, Cohen, and Smith (1950), however, have shown that pyrolusite is converted to Mn_2O_3 during the "catalytic" decomposition of hydrogen peroxide and Cowley and Walkley (1948) have found that the treatment of pyrolusite, with manganous chloride or perchlorate at a pH of 1, produces a surface layer which has a similar structure to goethite (α-FeOOH). Therefore, it is possible that a lower oxide of manganese, which is less reactive than the pyrolusite, is formed during reaction. A protective film of this type would have to explain two effects observed experimentally, firstly, the greater reaction rate in sulphate than perchlorate and secondly, the differences in shape of the rate curves in these two media. The following discussion is speculative but does offer an explanation of these results.

It is suggested that the lower oxide is formed rapidly and blocks the active sites in perchlorate solution resulting in a more uniform attack over the surface. In sulphate, however, adsorption of the anion may activate this material allowing the reaction to proceed unhindered along the crystal boundaries. Surface diffusion of ferrous ion has been suggested as the rate-determining step, and if this is so it is reasonable to suppose that the rate depends on the distance the ferrous ion has to travel to reach an active site, that is, the frequency of collision

between ferrous ions and active sites. This would account for the large difference in rates in the two media despite the difference in activation energy in the opposite sense.

In perchlorate solution the reaction rate progressively decreases (Fig. 6) and microscopic examination of the particles after partial reaction (Fig. 7 (*c*)) shows that the pitting is not as great as in sulphate solutions. After the initial 4 min the curves in Figure 6 correspond to a cube-root law which applies if the particles are spheres of continuously decreasing radii (Topley and Hume 1928). The 89-μ fraction which appears zero-order gives as good a straight line with the cube-root plot because the gradient is small. The rate curves in perchlorate therefore correspond to a rapid decrease in active surface in the first 4 min followed by a uniform attack over the whole surface of the particle.

V. Acknowledgments

The author wishes to express his thanks to Dr. A. Walkley of the Division of Industrial Chemistry, C.S.I.R.O., for his interest and advice in this work and to Mr. J. Woodcock of the Ore Dressing Laboratories, C.S.I.R.O., for infra-sizing the manganese dioxide samples.

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THE MECHANISM OF THE PINACOL–PINACONE REARRANGEMENT

VI. LOW TEMPERATURE KINETICS

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[*Manuscript received December* 6, 1956]

Summary

The low temperature rearrangement of pinacol to pinacone appears to be second order with respect to the hydrogen ion concentration. The activation energy is, however, similar to that of the high temperature reaction. This is interpreted as indicating that in the low temperature reaction the synartetic step is formed by a two stage process, through a stable intermediate, and each stage requires the participation of a hydrogen ion.

I. INTRODUCTION

The kinetics of this reaction in the temperature range 70–150 °C have been presented by Duncan and Lynn (1956*a*, 1956*b*, 1956*c*). They are in accordance with the conventional mechanism for the rearrangement (Ingold 1953). Recent work (Duncan and Lynn 1956, 1957) has however suggested that the reaction is more complicated than previously expected, especially below 70 °C. We now report kinetic measurements at low temperatures. All experimental methods, including preparation and standardization of reagents, were as previously described.

II. RESULTS

The first-order rate constants at temperatures of 60 and 50 °C, and those in acid-salt mixtures are given in Tables 1 and 2.

TABLE 1

FIRST-ORDER RATE CONSTANTS IN ACID-SALT MIXTURES AT 60 °C

[HCl] (mole/l)	[NaCl] (mole/l)	$k_{exp.}$	Expected Values of $k_{exp.}$ (sec^{-1}) for Pure HCl	
			At Same Concentration	At Same Ionic Strength
(1) 0·75	0·5	$1·6\times10^{-7}$	$1·65\times10^{-6}$	4×10^{-6}
(2) 0·50	1·0	$<10^{-9}$	$7·0\times10^{-7}$	5×10^{-6}

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TABLE 2

FIRST-ORDER RATE CONSTANTS ($k_{exp.}$)

Concentration in mole/l; $k_{exp.}$ in 10^5 sec^{-1}

$[H_2SO_4]$	$k_{exp.}$	[HCl]	$k_{exp.}$	$[HNO_3]$	$k_{exp.}$	[*p*-CH_3.C_6H_4.SO_3H]	$k_{exp.}$	$[HClO_4]$	$k_{exp.}$	$[(COOH)_2]$	$k_{exp.}$	$[H_3PO_4]$	$k_{exp.}$
At 60 °C													
1·14	0·35	0·99	0·31	1·07	0·33	0·89	0·24	1·05	0·28				
0·67	0·20	0·82	0·23	0·79	0·22	0·69	0·14	0·85	0·17				
0·39	0·065	0·63	0·14	0·40	0·071	0·33	0·045	0·70	0·13				
0·31	0·027	0·42	0·054	0·30	0·036			0·62	0·081				
		0·30	0·032										
At 50 °C													
7·2	28·81	1·14	0·064	1·07	0·043	0·89	0·037	1·57	0·18	0·51	0·0081	0·89	0·0047
6·6	11·38	0·73	0·030	0·79	0·036	0·69	0·022	1·03	0·11	0·41	0·0070	0·70	0·0019
4·9	3·18	0·61	0·014	0·48	0·014	0·51	0·011	0·68	0·035	0·31	0·0054	0·59	0·0014
2·87	0·70	0·47	0·012	0·40	0·0063	0·33	0·0069	0·46	0·0011	0·23	0·0032	0·42	0·0006
1·34	0·114							0·31	0·0084				
0·66	0·035												

III. Discussion

(a) *Kinetic Rate Equation*

It will be seen from Figure 1 that $k_{exp.}$ is not dependent on the first power of the hydrogen ion concentration, as it is at higher temperatures. Whilst some differences between the unibasic acids are to be expected at the higher concentrations, it is clear that at low acid concentrations, the second power of the hydrogen ion concentration is more reasonable. To eliminate the

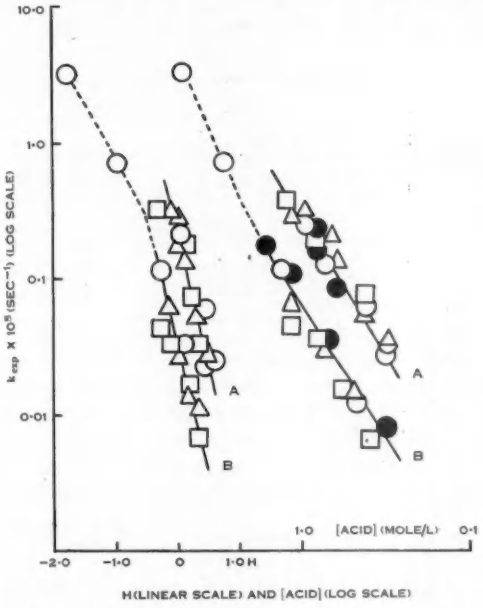

Fig. 1.—Variation of experimental rate constant with (i) acid concentration (log scale) and (ii) the Hammett acidity function H (linear scale).

A, 60 °C; *B*, 50 °C; ○ H_2SO_4; △ HCl; ● $HClO_4$; □ HNO_3.

possibility that the anion is taking part in the reaction, the results of Table 1 were determined. If the anion were involved, the reaction rate would be expected to rise significantly in the presence of the sodium salt of the catalysing acid, whereas in fact a large decrease in rate was observed. Likewise, a decrease in rate with weak acids would be expected if the hydrogen ion were the sole catalysing species (see Table 1). Apparently, then, in the temperature range 50–70 °C, the rate equation is

$$d[P]/dt = -k_1[P][H^+]^2,$$

where [P] is the pinacol concentration. Deviations at high acid concentrations in the plots of log $k_{exp.}$ *versus* (i) log [H^+] and (ii) the acidity function H are

similar to those obtained by Bell, Dowding, and Noble (1956) for a different reaction. In such cases one cannot be certain whether it is the hydrogen ion concentration or its activity which enters into the kinetic rate equation, although Bell, Dowding, and Noble (1956) favour the former.

(*b*) *Activation Energy*

The values of k_1 determined as described earlier (Duncan and Lynn 1956*a*, 1956*b*, 1956*c*) are $3{\cdot}0\times10^{-6}$ at 60 °C and $7{\cdot}0\times10^{-7}$ sec^{-1} at 50 °C. If one plots log k_1 *versus* $1/T$ it emerges, surprisingly, that the high temperature reaction and the low temperature reaction almost lie on the same straight line—in spite of the fact that the former involves the first power of the hydrogen ion concentration and the latter the second power. Hence, the high and low temperature reactions involve almost the same activation energy. Closer inspection reveals that there may be a small difference between the activation energies, which difference is close to the limits of experimental detection. A summary of the data is given in Table 3. The values quoted under " mean " refer to those already presented in Parts I–III of this series (Duncan and Lynn 1956*a*, 1956*b*, 1956*c*), which were taken from the best line through points above 60 °C. The values now reported at 50 and 60 °C suggest that there may have been a small error in the previously reported value, especially as the rate constant at 67·8 °C was determined with a single acid only ($HClO_4$).

TABLE 3

VALUES OF $\Delta H‡$, $\Delta S‡$

	$\Delta S‡$ (kcal/mole)	$\Delta S‡$ (cal/°C)
Mean values	31·5	36
High temp. reaction	35·0	46
Low temp. reaction	29·9	32

(*c*) *Mechanism of the Low Temperature Reaction*

The following data are now available on the low temperature reaction :

(i) There is no carbon-isotope effect, whereas there is a very large carbon-isotope effect for the high temperature reaction (Duncan and Lynn 1957).

(ii) The reaction proceeds via a stable intermediate (Duncan and Lynn 1957), which under some conditions can revert to pinacol (Duncan and Lynn 1957, unpublished data).

(iii) The reaction rate is dependent on the second power of the hydrogen ion concentration, at least at low acid concentrations.

It is clear that the reaction at high and at low temperatures has a similar activation energy. This is reassuring in that it is hardly possible to imagine a pinacol rearrangement occurring without a transition state similar to the synartetic ion. We therefore believe that the correct conclusion to be drawn

from this work is that the transition state arises in a different way from that in the high temperature reaction. The stable intermediate could be one of the following: (*a*) tetramethyl ethylene oxide, or (*b*) 2,3-dimethyl-1-butane-3-ol (found to form both pinacol and pinacone under the conditions of the reaction), or (*c*) the enolic form of pinacone. (Pinacone may be quantitatively recovered from the reaction mixture, which absorbs radiation at ~225 mμ (olefinic) and ~275 mμ (alcoholic). The absorption spectrum of pinacone itself with a peak at ~240 mμ (ketonic) reverts to the above on standing in acid even at room temperature.) We shall shortly discuss these features and the roles of these isomers in detail (Duncan, Kepert, and Lynn, unpublished data).

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REACTIONS OF AROYL PEROXIDES

III. BENZOYL PEROXIDE WITH CHLOROBENZENE, NAPHTHALENE, AND NITROBENZENE

By B. M. LYNCH* and K. H. PAUSACKER†

[*Manuscript received October* 16, 1956]

Summary

Quantitative product studies under varying conditions are reported for the systems named in the title to the present paper. The results obtained lend further support to our previous conclusion that benzoyl peroxide is not a suitable reagent for evaluation of reactivities towards free radicals (Pausacker 1957). Isomer ratios in the (mono-) phenylation and benzoyloxylation of naphthalene by benzoyl peroxide are reported and discussed.

I. INTRODUCTION

The present paper reports the results obtained from quantitative studies of the reactions of some simple aromatic compounds (chlorobenzene, naphthalene, and nitrobenzene) with benzoyl peroxide. These systems have been investigated previously by several groups of workers, but as far as we are aware, no complete

TABLE 1

REACTION PRODUCTS FROM SOME AROMATIC COMPOUNDS AND BENZOYL PEROXIDE

X in PhX	Temp. (°C)	Molar Concn.	CO_2	Reaction Products			
				$Ph.CO_2H$*		$Ph.C_6H_4X$*	High Mol.-Wt. Material† (%)
				1st	2nd		
OMe	100	0·2	0·51	1·22	—	0·36	—
H	78	0·4	1·19	0·45	0·31	0·40	30
Cl	80	0·12	1·30	0·50	0·13	0·54	30
NO_2	100–125	0·3	1·01	0·74	0·11	0·65	3
OH	60	0·05	0·02	1·60	0·26	Nil	—
Me	75	0·6	1·20	0·80	—	0·46	28

* Yields in mol/mol peroxide.

† Based on weight of benzoyl peroxide.

evaluations of yields of reaction products have been made. However, previous work does establish that the relative proportions of the various types of reaction products are markedly sensitive to the nature of the aromatic compound. This

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is shown in Table 1, where the yields of carbon dioxide, 1st benzoic acid,* 2nd benzoic acid,* substituted diphenyls, and high molecular-weight material are compared for the reactions of benzoyl peroxide with anisole (Suehiro 1951), benzene (Lynch and Pausacker 1957, Part I of this series), chlorobenzene (Barnett and Vaughan 1947 ; Roitt and Waters 1952 ; Augood, Hey, and Williams 1953), nitrobenzene (Bartlett and Altschul 1945 ; Barnett and Vaughan 1947 ; Hey, Nechvatal, and Robinson 1951 ; Hey, Stirling and Williams 1954), phenol (Suehiro 1951), and toluene (Dannley and Zaremsky 1955 ; Hey, Pengilly, and Williams 1955, 1956 ; Rondestvedt and Blanchard 1955).

In view of these large variations in relative yields, the present work was undertaken with the object of elucidating the effects of different aromatic compounds on the complete stoichiometry of the reactions.

II. Experimental

Melting points are corrected. Analyses are by the C.S.I.R.O. Microanalytical Laboratory.

(*a*) *Reagents*

Benzoyl peroxide, purified as in Part I of this series, has a minimum purity of 97 per cent. (iodometric analysis, Skellon and Wills 1948). Chlorobenzene was washed with concentrated sulphuric acid, then with aqueous sodium hydrogen carbonate, and finally with water. It was dried ($CaCl_2$) and distilled through a vacuum-jacketed Widmer column (b.p. 132 °C). Naphthalene was distilled (b.p. 219 °C, m.p. 80 °C). Nitrobenzene was shaken with aqueous sodium hydroxide, steam-distilled, dried ($CaCl_2$), and distilled (b.p. 212 °C). Benzoic acid, recrystallized from ethanol, had m.p. 122 °C. *o*-, *m*-, and *p*-Nitrophenols had m.p.'s 45, 97, and 114 °C. 2,6-Dibromo-4-nitrophenol, m.p. 142 °C (from aqueous acetic acid), was prepared by bromination of *p*-nitrophenol in acetic acid (Hartman and Dickey 1935). After purification by chromatography on alumina, samples of 1- and 2-naphthols had m.p.'s 95 and 122 °C. 4,4′-Diphenyldiphenyl (quaterphenyl), m.p. 312 °C (U.V. absorption : $\varepsilon_{max.}$ at 298 mμ : 39,000), was obtained from reaction of benzoyl peroxide with benzene (Part I). 1-Naphthoyl and 2-naphthoyl peroxides were prepared by Kharasch and Dannley's (1945) method. Although the m.p.'s of these compounds (98 and 138 °C respectively) agree with those recorded in the literature (Kharasch and Dannley 1945 ; Hey and Walker 1948), iodometric analyses indicated only 30 and 70 per cent. peroxide content. 1-Phenylnaphthalene, b.p. 146 °C/0·25 mm, and 2-phenylnaphthalene (plates, m.p. 103·5 °C, from ethanol) were prepared by reaction of the appropriate naphthoyl peroxide with benzene (cf. Hey and Walker 1948).

(*b*) *General Procedure*

Reactions were carried out, and the various products separated and identified, following the method outlined previously in Part I. Some minor modifications are described in the footnotes of Tables 2 to 4.

* For definitions of these terms see Part I of this series by Lynch and Pausacker (1957).—*Aust. J. Chem.* **10** : 40.

(c) *Results*

The results obtained are summarized in Tables 2 to 4. The values in parentheses are average yields in mol/mol of benzoyl peroxide. Further investigations on some of the reaction products are presented in the footnotes accompanying the tables. Unless otherwise stated, reactions were carried out at 78 °C.

TABLE 2

REACTIONS OF CHLOROBENZENE WITH BENZOYL PEROXIDE

Experiment No.:	11, 12	13	14	15	16,¶ 17¶	18
$(Ph.CO_2)_2$ (g) ..	6	10	6	5	7	5
PhCl (ml) ..	200	300	300	40	160	115
Time of heating (hr)	22, 21	19	27	14	2	2
Volume CO_2 (ml)	597, 709 (1·18)	1175 (1·27)	584 (1·06)	n.d.	n.d., 900 (1·38)	n.d.
1st $Ph.CO_2H$ (g)	1·67, 1·57 (0·56)	2·96 (0·59)	1·70 (0·58)	1·42 (0·56)	2·00, 2·00 (0·57)	1·40 (0·56)
2nd $Ph.CO_2H$ (g)	0·40, 0·40 (0·13)	0·87 (0·17)	0·40 (0·13)	0·37 (0·15)	0·1, 0·1 (0·03)	0·05 (0·02)
Phenols (g)* ..	0·14, 0·17 (0·06)	0·21 (0·04)	0·10 (0·03)	n.d.	0·05, 0·05 (0·02)	0·05 (0·02)
Chlorodiphenyls (g)†	2·12, 2·20 (0·46)	3·40 (0·44)	2·20 (0·47)	n.d.	2·85, 3·10 (0·54)	2·00 (0·51)
Quaterphenyl (g)‡	0·37, n.d. (0·05)	0·35 (0·03)	0·30 (0·04)	0·12 (0·02)	n.d.	n.d.
High-boiling residue (g)	2·14§, 2·15	3·31	2·10||	n.d.	3·55,⊥ 3·50⊥**	2·30⊥**

* Assumed to be isomeric chlorophenols.

† B.p. 115–130 °C/0·15 mm (Found: C, 76·0; H, 5·3%. Calc. for $C_{12}H_9Cl$: C, 76·4; H, 4·8%).

‡ This separated as the ether- and ethanol-insoluble material from the hydrolysed reaction mixtures (see Part I). M.p. 312 °C (from benzene) (Found: C, 94·0; H, 6·0%. Calc. for $C_{24}H_{18}$: C, 94·1; H, 5·9%).

§ Found: C, 79·9; H, 5·8; Cl, 14·2%; mol. wt. (Rast), 338. U.V. absorption: $\varepsilon_{max.}$ at 286 mμ: 11500 (calc. for mol. wt. = 338). A portion of this residue was dehydrochlorinated with palladized charcoal: The material (0·97 g) was heated (8 hr at 280–300 °C) with palladized charcoal (0·40 g) in a stream of pure hydrogen until evolution of hydrogen chloride ceased, and the mixture was extracted with benzene under reflux. Evaporation of the benzene yielded a gum (0·54 g), which possessed a brilliant blue fluorescence in ultraviolet light. Sublimation at 150–170 °C/15 mm yielded a trace of material, m.p. 180–190 °C, thought to be impure *p*-diphenylbenzene. Further sublimation at 200–250 °C/15 mm yielded 4,4′-diphenyldiphenyl (0·22 g), m.p. 309 °C (Found: C, 94·1; H, 5·8%) and mixed m.p. 309–310 °C.

|| Found: C, 81·4, 81·5, 80·2, 80·8; H, 5·5, 5·5, 5·5, 5·4; Cl, 12·7, 12·8%.

¶ These reactions at 132 °C.

⊥ Residues may contain amounts of unhydrolysed esters etc. as hydrolysis does not appear quantitative.

** Residues from these experiments were combined; an homogenized portion was analysed (Found: C, 74·8; H, 5·6; Cl, 15·2%). Fractional distillation yielded the following: 1·3 g, b.p. 110–160 °C/0·4 mm (Found: C, 77·2; H, 5·5; Cl, 14·9%); 1·5 g, b.p. 160–234 °C/0·4 mm (Found: C, 78·6; H, 5·3; Cl, 15·6%); 1·4 g, b.p. 240–260 °C/0·4 mm (Found: C, 79·7; H, 5·3; Cl, 13·8%); 0·8 g, b.p. 260–300 °C/0·4 mm (Found: C, 80·6; H, 5·4; Cl, 11·0%)

(*d*) *Ratios of Isomers Formed by Phenylation and Benzoyloxylation of Naphthalene*

(i) *Phenylation.*—The mixtures of phenylnaphthalenes isolated from experiments 19 and 22 were analysed by a standard ultraviolet spectrophotometric method. A Unicam S.P. 500 spectrophotometer was used, and calibration spectra of 1- and 2-phenylnaphthalenes in *cyclo*hexane (B.D.H. "Spectroscopic" grade) were recorded in the wavelength range 210–340 mμ. The

TABLE 3

REACTIONS OF NAPHTHALENE WITH BENZOYL PEROXIDE*

Experiment No.:	19	20	21,¶ 22
$(Ph.CO_2)_2$ (g)	6	9	20
$C_{10}H_8$ (g)	200	300	200
Time of heating (hr)	3·7	19	3·3, 8·5
Volume CO_2 (ml)†	233 (0·42)	n.d.	·757, n.d. (0·41)
1st $Ph.CO_2H$ (g)	2·80 (0·93)	4·30 (0·95)	9·94, 9·30 (0·96)
2nd $Ph.CO_2H$ (g)	1·03 (0·34)	1·90 (0·42)	3·08, 4·60 (0·39)
Naphthols (g)‡	0·64 (0·18)	1·30 (0·24)	3·25, 3·30 (0·28)
Phenylnaphthalenes (g)§ ..	2·00 (0·40)	4·40 (0·57)	n.d., 8·60 (0·51)
Diphenylnaphthalene (g)‖ ..	0·35 (0·05)	0·60 (0·06)	n.d., 1·30 (0·06)
High-boiling residue (g) ..	1·00	1·50	n.d., 3·00

* Benzene was added to the reaction mixtures after heating was stopped, to facilitate extractions. All reactions were carried out at 100 °C.

† These values are not very accurate, as it was not practicable to presaturate the solvent.

‡ Found: C, 82·8; H, 5·6%. Calc. for $C_{10}H_8O$: C, 83·3; H, 5·6%.

§ B.p. 140–200 °C/0·4 mm (Found: C, 94·1; H, 6·5%. Calc. for $C_{16}H_{12}$: C, 94·1; H, 5·9%).

‖ B.p. 200–250 °C/0·4 mm; crystallized from *cyclo*hexane, it had m.p. 133 °C (Found: C, 94·1; H, 5·5%; mol. wt. (Rast), 261. Calc. for $C_{22}H_{16}$: C, 94·3; H, 5·7%; mol. wt., 280). Allen and Gilman (1936) record m.p. 133 °C for 1,4-diphenylnaphthalene.

¶ In this experiment, the phenylated materials were not distilled, but 2-phenylnaphthalene, m.p. 102 °C, was isolated from the reaction mixture after fractional crystallization from ethanol; oxidation of the residue with alkaline potassium permanganate yielded *o*-benzoylbenzoic acid, which after drying at 100 °C had m.p. and mixed m.p. 128 °C.

concentrations of the solutions used were: 1-phenylnaphthalene, $2·62 \times 10^{-5}$; 2-phenylnaphthalene, $2·31 \times 10^{-5}$ mole/l. The spectra agreed excellently with those recorded previously (Friedel and Orchin 1951, spectra Nos. 290, 297). Recently-distilled samples of the phenylnaphthalene mixtures were used for the measurements of spectra. Homogenized samples were taken, and *cyclo*hexane was used as solvent; the concentrations used were $2·95 \times 10^{-5}$ mole/l. The extinction coefficients of the two mixtures were virtually identical, so the mean was used for the calculations. The percentages of 1- and 2-phenyl-

naphthalene were determined by comparing the ultraviolet spectra of the mixtures with the spectra of the pure isomers. The mean percentage composition was found to be: 1-phenylnaphthalene, 90; 2-phenylnaphthalene, 10. Over the wavelength range 210–340 mμ, the spectra of the mixtures were additive functions of these percentages of the two isomers.

(ii) *Benzoyloxylation.*—The mixture of naphthols isolated from experiment 20 was analysed by the method employed above for the phenylnaphthalenes. A Hilger Uvispek spectrophotometer was used, and calibration spectra of

TABLE 4

REACTIONS OF NITROBENZENE WITH BENZOYL PEROXIDE*

Experiment No.:	23, 24	25,† 26†	27, 28
$(Ph.CO_2)_2$ (g)	6	6	60
$Ph.NO_2$ (ml)	200	200	300
Time of heating (hr) ..	15, 16	4, 4	12, 12
Volume CO_2 (ml)	499, 532	560, 538	n.d.
	(0·93)	(0·99)	
1st $Ph.CO_2H$ (g)	2·05, 2·08	2·22, 2·20	21·2, 21·8
	(0·69)	(0·73)	(0·71)
2nd $Ph.CO_2H$ (g)	0·59, 0·60	0·55, 0·60	3·90, 3·44
	(0·20)	(0·19)	(0·12)
Phenols (g)	0·28, 0·28	0·28, 0·33	2·55,‖ 2·70
	(0·07)	(0·09)	(0·08)
Nitrodiphenyls (g)	3·28, 3·18	3·21, 3·25	17·2, 17·4
	(0·65)	(0·66)	(0·51)
High-boiling residue (g) ..	1·16,‡ 1·14	1·10, 0·92§	11·8,¶ 11·4

* After extraction of 1st benzoic acid, and removal of the excess of solvent, the reaction mixtures were hydrolysed by heating (8 hr) under reflux with 20% aqueous sodium hydroxide (200 ml). 2nd Benzoic acid and phenolic products were separated by standard methods. The isomeric nitrodiphenyls were separated from the residual nitrobenzene and high-boiling material by steam-distillation. It has already been shown (De Tar and Scheifele 1951) that this procedure gives a quantitative recovery of nitrodiphenyls.

† These experiments at 100 °C.

‡ The residue was chromatographed on alumina using benzene as eluant. Evaporation of the eluate yielded an orange-red semi-solid (Found: C, 80·7; H, 6·5; N, 3·9%).

§ Dehydrogenation was attempted using tetrachloro-1,4-benzoquinone as hydrogen acceptor (cf. Braude, Jackman, and Linstead 1954); the quinone was reduced, but no crystalline dehydrogenation products could be isolated.

‖ This fraction was dissolved in benzene and chromatographed on acid-washed alumina. Three bands were obtained: pale yellow, bright orange, and yellow-green. Elution of the pale yellow band with benzene, and evaporation of the eluate, yielded an oil, which on bromination gave 2,4,6-tribromophenol (0·17 g), m.p. and mixed m.p. 90–93 °C. Extrusion of the bright orange band, and extraction with ethanol (Soxhlet), followed by evaporation of the extract, gave *o*-nitrophenol (1·64 g), m.p. and mixed m.p. 45 °C. Extrusion of the yellow-green band, and extraction with 5N ethanolic hydrochloric acid, gave impure *p*-nitrophenol (0·47 g), m.p. 105–115 °C, on evaporation of the extract. Bromination in acetic acid yielded 2,6-dibromo-4-nitrophenol, m.p. and mixed m.p. 142 °C.

¶ Found: C, 75·9; H, 5·1; N, 4·6; O, 14·6%. Chromatography on alumina, elution with benzene, and evaporation of the eluate yielded an orange semi-solid (Found: C, 77·6; H, 4·9; N, 5·2%).

1- and 2-naphthols were recorded in the wavelength range 220–340 mμ, using absolute ethanol (B.D.H. "Spectroscopic" grade) as solvent. The concentrations used were: 1-naphthol, $3{\cdot}61\times10^{-5}$; 2-naphthol, $3{\cdot}40\times10^{-5}$ mole/l. The spectra agreed excellently with those recorded previously (Friedel and Orchin 1951, spectra Nos. 236, 237). The mixture of naphthols (1·05 g) was chromatographed on alumina, and the naphthols were eluted with benzene (400 ml). The solvent was evaporated under reduced pressure, and the spectrum of the homogenized mixture was recorded, using absolute ethanol as solvent. The concentration of the solution was $3{\cdot}38\times10^{-5}$ mole/l. The percentages of 1-naphthol and 2-naphthol, determined by comparing the ultraviolet spectrum of the mixture with the spectra of the pure isomers, were: 1-naphthol, 88; 2-naphthol, 12. Over the wavelength range 220–340 mμ, the spectrum of the mixture was an additive function of these percentages of the two isomers.

III. Discussion

(*a*) *General*

Balanced equations can be derived for all these reaction systems using a scheme similar to that outlined in Part I of this series (Lynch and Pausacker 1957). The equations will not be given in detail but some representative results are presented in Table 5. Detailed equations have been given recently by Lynch (1956).

Table 5

Reactions of benzoyl peroxide with chlorobenzene, naphthalene, and nitrobenzene

	Aromatic Compounds		
	Chlorobenzene	Naphthalene	Nitrobenzene
Experiment No.:	11–13	19, 21, 22	25, 26
Yields:*			
Sum of CO_2, 1st and 2nd $Ph.CO_2H$ (mol)	1·99	—	1·92
Substituted diphenyls (mol)	0·49	0·40	0·66
Substituted phenols (mol)	0·06	0·28	0·09
Mol. aromatic compound reacting*	0·91	0·91	0·94†
High-boiling residue (mol): *			
Ph	0·72	0·11	0·37
$Ph.CO_2$	Nil	0·18	0·11
Aromatic compound	0·30	0·21	0·15
Substituent accounted for (%) ..	103	—	99

* Per mol of benzoyl peroxide.

† Assuming yield of carbon dioxide as 0·66 mol.

An accurate assessment of products has most probably been made, since the sum of the yields of carbon dioxide and the 1st and 2nd benzoic acids is fairly close to 2 mol for the various systems; further, the yields of the other products were quite reproducible. The effects of increasing peroxide concentration and temperature of reaction are similar to those observed with benzene

(Part I of this series; also see McClure, Cuthbertson, and Robertson 1942) in that the yields of carbon dioxide increase with increasing temperature and with decreasing peroxide concentration. General comparisons between the three systems studied in the present paper and the benzene-benzoyl peroxide system (Part I) reveal that although approximately 1 mole of each aromatic compound reacts with 1 mole of benzoyl peroxide, the relative yields of the various products are markedly influenced by the aromatic compound (cf. Table 1).

Thus the average molar yields of carbon dioxide, 1st benzoic acid, substituted diphenyls, and substituted phenols (formed by benzoyloxylation), respectively, found for the various systems are: (i) benzene (Part I): 1·19, 0·45, 0·40, nil; (ii) chlorobenzene: 1·27, 0·55, 0·46, 0·06; naphthalene: 0·42 (0·66), 0·95, 0·40–0·45, 0·28; nitrobenzene: 1·00, 0·73, 0·65, 0·09. These results lend further convincing support to our previous contention that the so-called " competitive " method of evaluating reactivities by direct comparison of the yields of substituted diphenyls formed in mixed systems is only approximate. This is readily seen when it is realized that the validity of this method depends upon the truth of the assumption that the substances being compared undergo stoichiometrically identical reactions with the substituting agent. This assumption is valid for nitration, but obviously not for the processes occurring when benzoyl peroxide reacts with aromatic compounds. Hey (1952) has assumed that the principal reaction occurring between benzoyl peroxide and an aromatic compound is

$$(Ph.CO_2)_2 + Ar.H \rightarrow Ar.Ph + Ph.CO_2H + CO_2, \quad \ldots\ldots\ldots \quad (1)$$

with reaction (2) as a minor side-reaction

$$(Ph.CO_2)_2 + Ar.H \rightarrow Ar.O.C(:O)Ph + Ph.H + CO_2. \quad \ldots\ldots \quad (2)$$

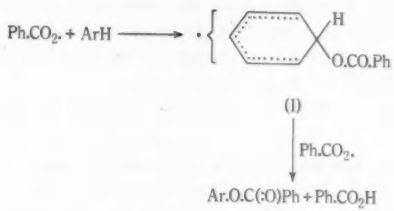
The present work shows that reaction (1) accounts for a maximum of 65 per cent. of the benzoyl peroxide used; further, to our knowledge, no evidence for the occurrence of reaction (2) has ever been presented although it does take place when aliphatic C–H bonds are present.

Furthermore, in the series benzene, chlorobenzene, nitrobenzene, naphthalene, the ratio of benzoyloxylated products to phenylated products is 0·00, 0·13, 0·14, and 0·67, respectively. The degree of benzoyloxylation of the compounds studied is obviously variable and so should not be disregarded when relative reactivities are being considered (see also Section III (*d*)).

(*b*) *Mechanisms of Product Formation*

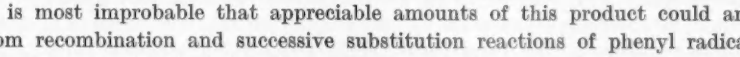
Most of these have been discussed in Part I. However, the formation of benzoyloxylation products has not been explained. It is thought that benzoyloxylation of an aromatic compound occurs by a mechanism analogous to that of diphenyl formation, that is, reaction proceeds by initial addition of a benzoyloxy-radical to the aromatic compound to yield the mesomeric addition product I; conversion into an aryl benzoate is effected by disproportionation

with another radical, very probably another benzoyloxy-radical (cf. Szwarc 1953), that is,



Confirmation of this suggestion is afforded since the sum of the yields of substituted diphenyls and aryl benzoates is equal, within experimental error, to the yield of 1st benzoic acid.

The mechanism of formation of 4,4′-diphenyldiphenyl (quaterphenyl) in the reaction between chlorobenzene and benzoyl peroxide is also of interest. It is most improbable that appreciable amounts of this product could arise from recombination and successive substitution reactions of phenyl radicals, and it is suggested that it is formed by dimerization and subsequent oxidation of an adduct between phenyl radical and chlorobenzene, just as in the reaction between benzene and benzoyl peroxide (Part I). To account for the dechlorination of the dimerized adduct, it is assumed that reaction involves attack at the carbon atom bearing the substituent, that is,



Formation of benzoic anhydride in this reaction is believed to account for the differing yields of 2nd benzoic acid (0·13–0·17 mol) and of chlorophenols (0·04–0·06 mol). This mechanism of formation of quaterphenyl is purely tentative and a referee has suggested that at least part of the quaterphenyl may be formed by the reaction of III with the alcoholic potassium hydroxide used in the hydrolysis procedure. Investigation of the high-boiling materials isolated in the chlorobenzene-benzoyl peroxide reactions indicated that these contained appreciable amounts of quaterphenyl derivatives, with only traces of terphenyls (see Section II); these results are in contrast to previous suggestions (Barnett and Vaughan 1947; Roitt and Waters 1952; Augood, Hey, and Williams 1953) that the high-boiling material consists largely of chloroterphenyls, presumably formed by successive substitution.

(c) *Free-Radical Substitution of Naphthalene*

Boyland and Sims (1953) have shown that 1- and 2-naphthyl benzoates are formed on reaction of benzoyl peroxide with naphthalene in chlorobenzene solution at 80 °C (cf. Roitt and Waters 1952). Huisgen and Sorge (1950) found that both 1- and 2-phenylnaphthalene were formed by reaction of *N*-nitrosoacetanilide with naphthalene. Using the fact that only the 2-isomer forms a 2,4,7-trinitrofluorenone derivative, they arrived at a ratio of 1- to 2-substitution of 5 : 1. Dannley and Gippin (1952) have investigated the reactions between benzoyl peroxide and some substituted naphthalenes; they found that benzoyloxylation occurred to a large extent (to 0·62 mol), but no evolution of carbon dioxide, or phenylation of the naphthalenes, took place. These results are difficult to reconcile with those obtained in the present work, where concurrent phenylation and benzoyloxylation is observed. We have determined the ratios of 1- to 2-substitution in both the phenylation and benzoyloxylation of naphthalene by benzoyl peroxide, using ultraviolet spectrophotometry (see Section II). The results show that for both phenylation and benzoyloxylation, the ratio of 1- to 2-substitution is approximately 9 : 1. 1,4-Diphenylnaphthalene was also isolated in these reactions, and as this is assumed to be formed by further phenylation of 1-phenylnaphthalene (cf. formation of *p*-terphenyl in the corresponding reaction with benzene, Part I of this series), the proportion of isomers should, strictly speaking, be corrected for the amount of 1-phenylnaphthalene consumed in this further reaction; however, this correction does not appreciably alter the substitution ratio. The greater reactivity at the 1-position for substitution by phenyl and by benzoyloxy-radicals is in qualitative agreement with Wheland's (1942) predictions derived from calculations using the molecular-orbital approximation. However, the calculations imply that the ratio of 1- to 2-substitution should be much greater than is actually observed as a substitution ratio of 10^3 : 1 is predicted. Similar discrepancies have been noted for the electrophilic substitution of naphthalene (Bavin and Dewar 1956) (e.g. nitration (Dewar and Mole 1956), which gives a ratio of 1- to 2-substitution of from 8 : 1 to 10 : 1, depending on the reaction temperature). Bavin and Dewar (1956) have suggested that the disagreement arises because the structures considered by Wheland as transition states are stable intermediates; in the transition states, the attacking reagent is still remote and weakly bonded.

The 1- to 2-substitution ratios observed in our experiments also afford excellent confirmatory evidence for Dewar's (1952) prediction that the orientation of aromatic substitution reactions of alternant hydrocarbons is similar for electrophilic, nucleophilic, and free-radical reactions.

(d) *Comparative Reactivities of the Compounds Studied*

As the relative proportions of the reaction products from the various systems vary markedly with the different aromatic compounds (see Table 4), no quantitative assessment of their reactivities towards free radicals can be derived. However, comparisons of the yields of certain of the products obtained

in the various reaction systems enable a qualitative order of reactivities to be established.

The processes occurring when benzoyl peroxide reacts with an aromatic compound can be summarized as follows:

$$\mathrm{Ph.} + \mathrm{Ar.H} \longrightarrow \underset{\text{(IV)}}{\mathrm{Ph...Ar...H}} \xrightarrow{(a)} \mathrm{Ph.Ar.}$$

$$\xrightarrow{(b)} \text{Addition and polymerization products.}$$

$$\xrightarrow{(c)} \text{Dimerization products.}$$

$$\mathrm{Ph.CO_2.} + \mathrm{Ar.H} \longrightarrow \underset{\text{(V)}}{\mathrm{Ph.CO_2..Ar..H}} \xrightarrow{(d)} \mathrm{Ph.CO_2Ar.}$$

where processes (*a*) and (*d*) are the phenylation and benzoyloxylation reactions, with disproportionation between the adducts IV and V and benzoyloxy-radicals yielding substituted-diphenyls and aryl benzoates respectively. Process (*b*) involves further addition of a phenyl radical to the adduct IV, with subsequent polymerization; process (*c*) yields tetrahydroquaterphenyl derivatives. In Table 6, the molar amounts of the adducts IV and V consumed in the various reaction processes are compared for the reactions of benzoyl peroxide with benzene (Part I), chlorobenzene, naphthalene, and nitrobenzene.

TABLE 6

RELATIVE CONTRIBUTIONS OF DIFFERENT REACTION PATHS

Aromatic Compound	Amount Reacting (mol)	Ph. Formed	Adduct IV Reacting by Process			Adduct V Reacting by Process (*d*)
			(*a*)	(*b*)	(*c*)	
PhH	1·00	1·13	0·40	0·40	0·18	—
PhCl	0·91	1·27	0·46	0·30	0·06	0·06
$PhNO_2$	0·91	0·99	0·66	0·15	—	0·08
$C_{10}H_8$	0·94	(0·66)	0·45	0·11	—	0·28

Since the activation energies for all the processes (*a*) to (*d*) are very low (cf. Levy and Szwarc 1955), it is thought that the increasing importance of benzoyloxylation (process (*d*)) in the order benzene<chlorobenzene<nitrobenzene<naphthalene reflects increasing reactivity towards free-radical attack. With the more reactive compounds, fewer benzoyloxy-radicals undergo decomposition to yield the more reactive phenyl radicals (cf. the reactions of benzoyl peroxide with phenols, where the benzoyloxy-radicals react as such without appreciable decomposition (Cosgrove and Waters 1949; Suehiro 1951)), and less carbon dioxide is evolved. Further, the phenylation reaction (process (*a*)) accounts for increasing percentages of reaction of the adducts II in the order: benzene (40 per cent.), chlorobenzene (51 per cent.), naphthalene (81 per cent.),

nitrobenzene (82 per cent.). Thus it is evident, as Hey and Williams (1953) have suggested, that arylation becomes a more effective reaction path as the extent of conjugation in the parent molecule increases; this probably occurs because the hydrogen atom at the site of substitution is bound less strongly in the adducts II derived from the more highly-conjugated compounds. However, the increasing effectiveness of process (*a*) in the reactions of the adducts II does not imply that phenylation can eventually become the sole path of reaction, as it is seen that benzoyloxylation becomes more important, and the amount of available phenyl radicals decreases. Thus the yield of substituted diphenyls would be expected to pass through a maximum, as has been found in the reaction with nitrobenzene. For benzene and chlorobenzene, which are less reactive towards free radicals than nitrobenzene, addition and dimerization (processes (*b*) and (*c*)) are more important than with nitrobenzene; for naphthalene, which is more reactive than nitrobenzene, benzoyloxylation (process (*d*)) becomes more important.

Therefore, although no quantitative assignment of reactivities can be made, the reactivities of the aromatic compounds so far investigated are in the order: benzene<chlorobenzene<nitrobenzene<naphthalene. This order is in agreement with that suggested by Hey and Williams (1953) on the basis of "competitive" experiments. We believe that the order of reactivities established by these competitive experiments is probably correct, but little significance can be attached to the numerical values of the relative reactivities, which are often expressed to three significant figures, obtained by this method. It is also apparent that the partial rate factors are in the correct relative order as Hey, Stirling, and Williams (1955) have shown that the same isomer ratio is obtained in the phenylation of pyridine with benzoyl peroxide, lead tetrabenzoate, phenyl iodosobenzoate, nitrosoacetanilide, and phenylazotriphenylmethane. We have already commented (Pausacker 1957) that *m*- and *p*-nitrobenzoyl peroxides may be more useful in determining the order of reactivities. In view of the results obtained in the present paper, we intend to investigate the reaction of these two peroxides with the more reactive compounds (e.g. nitrobenzene and naphthalene) in order to determine whether benzoyloxylation is still an important simultaneous reaction.

In addition to the experiments reported in the present paper, we have carried out similar studies of the reactions of benzoyl peroxide with anisole, *p*-dichlorobenzene, diphenyl, anthracene, and phenanthrene. We have also investigated the reactions of phenyl iodosobenzoate with chlorobenzene, *p*-dichlorobenzene, anisole, and nitrobenzene. The results obtained with this latter reagent complement those recently published by Hey, Stirling, and Williams (1955, 1956) and will be presented in detail in a subsequent paper.

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THE ALKALOIDS OF *LUPINUS VARIUS* L.

II. ALKALOIDS OF THE LEAF

By W. D. Crow* and M. Michael*

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Summary

The aerial parts of *Lupinus varius* L. contain the alkaloids LV-1 (0·10 per cent.) and (+)-*epi*lupinine (0·50 per cent.) previously reported in the seeds, together with (—)-sparteine (0·006 per cent.) and small amounts of two mono-nitrogen bases LV-3 ($C_{20}H_{27}O_4N$, containing one methoxyl group) and LV-4 ($C_{17}H_{23}O_5N$). The proportion of LV-1 present in the leaves is higher than that in the seeds, while the amount of (+)-*epi*lupinine-*N*-oxide is much lower.

I. Introduction

The seeds of *Lupinus varius* L. (Western Australian "blue lupin") have been used in certain areas of Western Australia as sheep fodder, the seeds and dried plant only being consumed, since the animals refuse to eat the aerial portions of the plant except in the early stages of growth (Norris 1943). Heavy sheep losses in the 1949–51 seasons prompted an examination of the seeds for alkaloids, and two new bases, LV-1 ($C_{15}H_{22}ON_2$) and (+)-*epi*lupinine-*N*-oxide, were isolated in substantial amount (Crow and Riggs 1955). Although there was no direct evidence that the alkaloids were responsible for the deaths observed in the field, the high alkaloid content (1·25 per cent.) of the seeds made it desirable that these should be isolated and examined.

The fact that the young plants may be grazed with no ill-effects suggested that an examination of the aerial portions of the plant might reveal some differences in alkaloid content, and this has proven to be the case. The comparison between seeds and aerial parts is summarized in Table 1. The results unfortunately refer to material gathered in different seasons, but unless the species is subject to gross variation in alkaloid content the overall picture should be a representative one. The most striking feature is the reversal of the *epi*-lupinine/*N*-oxide ratio in passing from aerial parts to seeds, suggesting that this base may be preferentially stored in the form of the *N*-oxide. Another significant point is the higher proportion of base LV-1 present in the seeds; this compound is the most toxic of the major constituents.

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The absence of the minor constituents from the seeds is of little real significance, since the aerial parts were examined by an improved method. The significance of the variations in relation to the use of this plant as stock food is not clear, and must await proof that the alkaloids are in fact responsible for the losses incurred in the field.

TABLE I

ALKALOID CONTENT OF L. VARIUS

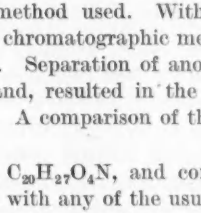
Compound	Percentage in Seeds*	Percentage in Leaf
(+)-*epi*Lupinine-*N*-oxide ..	0·94	0·25
(+)-*epi*Lupinine	0·13	0·50
Base LV-1	0·21	0·07
Base LV-3	—	0·02
Base LV-4	—	Trace only
(—)-Sparteine	—	0·01

* Crow and Riggs (1955).

II. Isolation of the Alkaloids

The free bases and *N*-oxides were separated from each other as described by Crow and Riggs (1955) in Part I of this series. Separation of the individual bases was achieved by two different methods, and slightly different results were observed according to the method used. With one batch of material the separation was essentially by chromatographic methods, and (—)-sparteine and base LV-4 were not detected. Separation of another batch by countercurrent distribution, on the other hand, resulted in the ready isolation of these two bases, but loss of base LV-3. A comparison of the two methods is included in Section III.

Base LV-3 analysed for $C_{20}H_{27}O_4N$, and contained one methoxyl group. These data are not consistent with any of the usual lupin type structures based on the quinolizidine nucleus, and it is considered that base LV-3 is more probably of the type exemplified by calycotomine (I) (White 1951). The ultraviolet



(I)

spectrum, which shows maxima at 235 mμ (log ε 3·81) and 282 mμ (log ε 3·33) in neutral or acid solution, is consistent with this interpretation. The amount of material isolated was inadequate for a more detailed examination of this alkaloid. In the case of base LV-4 only sufficient material was obtained for analysis, which resulted in a provisional formula $C_{17}H_{23}O_5N$.

III. Experimental

All melting points are corrected. Microanalyses were carried out by C.S.I.R.O. Microanalytical Laboratory, University of Melbourne, under the direction of Dr. K. W. Zimmerman. All R_F values were determined in a solvent consisting of *n*-BuOH : acetic acid : water in the proportions 80 : 3 : 17 by volume, using Whatman No. 1 paper. Light petroleum refers to a hydrocarbon fraction, b.p. 50–70 °C.

(*a*) *Extraction of the Tops.*—The milled aerial parts of the plant (10·9 kg) were exhausted with methanol, the extract concentrated, diluted with 5% hydrochloric acid (1 l.), and extracted several times with chloroform to remove non-basic material. The aqueous solution was then basified to pH 12 with 40% sodium hydroxide and extracted with chloroform (5 × 1 l.), each extract being washed successively with the same 5% sodium hydroxide (2 l.) to prevent removal of the *N*-oxide from the aqueous solution. Evaporation of the combined chloroform extracts afforded the free bases as an amber gum (85 g). The combined aqueous solutions were acidified and stirred with zinc dust to reduce the *N*-oxides (8 hr at 20 °C), and the bases generated were extracted with chloroform after basification to pH 12. Evaporation of the combined chloroform extracts afforded 28 g (+)-*epi*lupinine, m.p. 77–78 °C, after recrystallization, corresponding to an *N*-oxide content of 0·25%.

(*b*) *Separation of the Free Bases.*—(i) *By Chromatography.* The bases (25 g) from 4·5 kg plant material were dissolved in dry chloroform (200 c.c.) and chromatographed over alumina (activity I, 1·5 kg) in the same solvent. The following fractions were collected :

Fraction		R_F Values
A	2·0 g eluted by chloroform	*0·25*,* 0·34, 0·70
B	15·5 g eluted by $CHCl_3$/1–2% MeOH	0·23, *0·36*
C	2·2 g eluted by $CHCl_3$/2% MeOH	0·08, 0·14, 0·22, 0·32, 0·38
D	3·7 g eluted by $CHCl_3$/5–100% MeOH	0·10, *0·32*, 0·43

Fraction *A* was chromatographed again under the same conditions, and afforded base LV-1 (1·7 g) as colourless needles, m.p. 103–104 °C, from light petroleum. Later fractions from the column were partly mixtures, but a little (0·2 g) (+)-*epi*lupinine, m.p. 77–78 °C, was obtained.

Fraction *B* was similarly treated to give base LV-1 (1·4 g) and (+)-*epi*lupinine (12·8 g).

Fraction *C* failed to give any crystalline material after repeated chromatography, and was ultimately abandoned.

Fraction *D* was separated by partial solution in methanol into an oily mixture and a crystalline solid (1·06 g) of R_F 0·32. Crystallization from methanol afforded *base LV-3* as colourless needles, m.p. 232–233 °C (decomp.) (Found : C, 69·8 ; H, 8·1 ; O, 18·2 ; N, 4·3 ; CH_3O, 8·8%. Calc. for $C_{20}H_{27}O_4N$: C, 69·5 ; H, 7·9 ; O, 18·5 ; N, 4·1 ; 1 × CH_3O, 9·0%). The *picrate* crystallized from aqueous acetone as yellow needles which melted at 186 °C and decomposed sharply at 231 °C (Found : C, 54·4 ; H, 5·4 ; O, 30·6 ; N, 9·6%. Calc. for $C_{20}H_{27}O_4N.C_6H_3O_7N_3$: C, 54·4 ; H, 5·3 ; O, 30·6 ; N, 9·8%). Base LV-3 gave a negative Labat test for the methylenedioxy-group. The ultraviolet spectrum in ethanolic solution showed two peaks at 235 mμ ($\log \varepsilon$ 3·81) and 282 mμ (3·33), and was not altered by the addition of acid.

$E_1, E_2, E_3, \ldots\ldots\ldots\ldots\ldots, E_{24}, E_{25}$

$A_{25}, A_{24}, A_{23}, \ldots\ldots\ldots\ldots\ldots, A_3, A_2, A_1$

Fig. 1

(ii) *By Countercurrent Distribution.* The mixed bases (85 g) from 10·9 kg plant material were subjected to 25-plate distribution between ether and 2% sodium hydroxide solution (1·5 l. each phase). The state of the separation at the conclusion of this operation is shown diagrammatically in Figure 1.

* Major constituents are printed in italics.

The fractions were grouped according to the results of paper chromatography as follows:

Fraction		(g)	R_F Values
A	E_1–E_2	(2·2)	0·16, 0·34, *0·49*, 0·56
B	E_3	(0·3)	0·14, 0·37, *0·47*
C	E_4–E_{11}	(2·1)	0·14, *0·34*
D	E_{12}–E_{25}	(44·0)	0·35
E	A_{23}–A_{25}	(7·5)	0·23, *0·35*
F	A_{16}–A_{22}	(9·9)	*0·23*, *0·34*
G	A_{11}–A_{15}	(3·3)	0·24, 0·36
H	A_5–A_{10}	(2·7)	0·10, *0·24*, 0·33
J	A_1–A_4	(1·8)	0·10, *0·16*, *0·24*

Fraction *A* was dissolved in ether and extracted with 5% hydrochloric acid, the bases being recovered into ether (1·53 g). After standing for several days in a little acetone a small amount of crystalline material was obtained. This was recrystallized from chloroform/light petroleum as colourless needles, m.p. 235–237 °C (decomp.) (Found : C, 63·8 ; H, 7·5 ; N, 4·7%. Calc. for $C_{17}H_{23}O_5N$: C, 63·5 ; H, 7·2 ; N, 4·4%). The mother liquors were evaporated to dryness and the residue distilled under reduced pressure to give a colourless oil, b.p. 140 °C (bath)/0·5 mm (Found : C, 76·5 ; H, 10·8 ; N, 11·6%. Calc. for $C_{15}H_{26}N_2$: C, 76·9 ; H, 11·2 ; N, 12·0%) which had $[\alpha]_D^{20}$ —18·6° (*c*, 1·8 in ethanol). The picrate crystallized from ethanol in yellow needles, m.p. 207–208 °C, alone or in admixture with an authentic specimen of (—)-sparteine dipicrate. The base had R_F 0·51, as did authentic (—)-sparteine.

Fraction *B*, after removal of non-basic material, was found to consist almost entirely of sparteine, identified through the dipicrate.

Fraction *C*, after removal of non-basic material (1·0 g) was dissolved in light petroleum. After several days' standing some (+)-*epi*lupinine (0·5 g) was obtained as crystals, m.p. 77–78 °C, but the mother liquors proved to be intractable, and no further crystalline material was obtained.

Fraction *D* was fractionally crystallized from acetone and light petroleum to give a total of 37·6 g (+)-*epi*lupinine, m.p. 77–78 °C. The mother liquors (6·3 g) had R_F 0·12, *0·34*, 0·50, and consisted largely of the same base.

Fraction *E* was similarly crystallized from acetone and light petroleum to give a total of 7·0 g (+)-*epi*lupinine. The mother liquors had R_F *0·23*, *0·35*.

Fraction *F* was treated as for the preceding fraction, and after several days in acetone solution deposited large crystals (1·3 g) of (+)*epi*lupinine. The mother liquors were combined with those of fractions *D* and *E* (total=13·2 g) and chromatographed over alumina as described under Section III (*b*) (i). The products obtained were base LV-1 (2·3 g) and (+)-*epi*lupinine (8·3 g).

Fraction *G* was crystallized from light petroleum to give base LV-1 (0·6 g, m.p. 102–104 °C). The mother liquors were chromatographed over alumina in benzene, and afforded base LV-1 (1·35 g) and (+)-*epi*lupinine (0·72 g).

Fraction *H* was similarly treated to give a total of 1·7 g base LV-1.

Fraction *J*, after removal of the low R_F materials by passage through a short column of alumina in benzene, afforded only base LV-1 (1·3 g).

Summary of Separations

(1) By chromatography : 25 g bases gave 3·1 g base LV-1 (0·07%) ; 12·9 g (+)-*epi*lupinine (0·3%) ; and 1·06 g base LV-3 (0·02%).

(2) By distribution : 85 g bases gave 7·2 g base LV-1 (0·07%) ; 55·4 g (+)-*epi*lupinine (0·5%) ; 1·0 g (—)-sparteine (0·01%) ; and 0·3 g base LV-4 (trace).

It is clear that the distribution method is distinctly superior for the isolation of (+)-*epi*-lupinine, but neither method can be relied upon to give a good recovery of base LV-1. The total recovery values (68 and 75% respectively) indicate the amount of intractable oils which were abandoned during the separations.

IV. Acknowledgments

The authors are indebted to Mr. J. E. Peterson, Department of Agriculture, Western Australia, for the supply of plant material, and to Mr. P. Hunter, Division of Industrial Chemistry, C.S.I.R.O., for the preparation of the extracts.

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CHEMISTRY OF NON-ENZYMIC BROWNING

I. REACTIONS BETWEEN AMINO ACIDS, ORGANIC ACIDS, AND SUGARS IN FREEZE-DRIED APRICOTS AND PEACHES

By E. F. L. J. ANET* and T. M. REYNOLDS*

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Summary

The water-soluble constituents of apricot and peach purées were examined qualitatively and quantitatively before and after storage for 4 to 16 months at 25 °C and 70 per cent. R.H. All samples contained all the amino acids, organic acids, sugars, and polyols previously detected (Reynolds 1957; Anet and Reynolds 1955*a*, 1955*b*; Ash and Reynolds 1955*a*, 1955*b*) in the two species of fruit.

The stored samples also contained the following compounds: eleven 1-(*N*-amino acid)-1-deoxyfructoses, traces of two 2-(*N*-amino acid)-2-deoxyglucoses, nine by-products of unknown constitution formed from the reaction between glucose and aspartic acid and glucose and asparagine, three compounds formed from ammonia and glucose, two series of sucrose, glucose, and fructose mono-esters of malic acid, traces of a sorbitol mono-ester of malic acid, and some sugar mono-esters of citric acid.

The free amino acid or organic acid lost was equal, on a molar basis, to the amino acid–deoxyfructoses (and related compounds) and sugar esters formed. After allowing for these derivatives as much as 7 per cent. of the total sugar (calculated as hexoses) originally present could not be accounted for.

I. INTRODUCTION

The chemical reactions responsible for non-enzymic browning are complex and, even in model systems, only partially understood (see reviews by Danehy and Pigman 1950; Hodge 1953, 1955). In some foodstuffs the only reactants are aldoses and amino compounds (cf. Lea 1951), but in fruits fructose and organic acids are also involved. Haas and Stadtman (1949) prepared cationic, anionic, and neutral fractions from an apricot concentrate and showed that browning occurred when any two fractions were combined and stored at 57 °C, whilst Livingston (1953) found that a fructose-malic acid mixture browned in 48 hr at 60 °C but glucose and malic acid did not.

Detailed chemical studies have now been carried out on browned freeze-dried apricot and peach purées which had been stored for 4 to 16 months at 25 °C and 70 per cent. R.H. The nature of the primary ionic reaction products has been established and many of these products have been individually identified. Some of the quantitative changes occurring in amino acids, organic acids, and sugars during storage have also been determined. Some of these results have been described in a preliminary communication (Anet and Reynolds 1956).

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The soluble cationic and anionic constituents in 80 per cent. ethanolic extracts were collected on ion-exchange resins and fractionated by displacement chromatography (Partridge and Brimley 1952 ; Anet and Reynolds 1955*a*). De-ionized solutions containing the neutral constituents were examined by paper chromatography. All the amino acids, organic acids, sugars, and polyols previously found in apricots and peaches (Reynolds 1957 ; Anet and Reynolds 1955*a*, 1955*b* ; Ash and Reynolds 1955*a*, 1955*b*) were detected in the purées both before and after storage. The browned purées contained a large number of cationic (Table 1) and anionic (Table 3) constituents which were not present before storage and which were the same in both species of fruit. Faint traces of new compounds, which gave streaks on paper chromatograms, appeared amongst the neutral constituents of stored samples.

Most of the cationic constituents formed during storage were weak bases which emerged from the columns before aspartic acid (Table 1), although small quantities of stronger bases emerged (fractions 90–95, Table 1) just before ammonia. All these compounds reacted with ninhydrin but they were distinguished from the amino acids by their reaction with alkaline silver nitrate and triphenyltetrazolium chloride. This suggested that they were 1-(*N*-amino acid)-1-deoxyfructoses, the Amadori rearrangement products formed from the reaction between glucose and amino acids. A range of amino acid-deoxyfructoses was synthesized and comparisons of colour reactions and chromatographic behaviour, confirmed by the liberation of the parent amino acid with dilute acid, established the identity of F1–F11 (Table 2). Some of these compounds had been previously prepared by Gottschalk (1952) and by Abrams, Lowy, and Borsook (1955) who were unable to crystallize them. Preparation by another method and purification by displacement chromatography also gave amorphous products, with the exception of 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose which are described in Part II of this series (Anet 1957).

The colour reactions and chromatographic behaviour of nine other constituents (F1*a*–F1*e* and F4*a*–F4*d*, Table 1) of the browned fruit corresponded with those of synthetic by-products (Table 2) from the reaction of glucose with aspartic acid and asparagine. The structures of these compounds, which have not yet been obtained pure, are unknown but the properties of F1*d* and F4*d* suggest that they are lactones of aspartic acid-deoxyfructose and asparagino-deoxyfructose. From similar comparisons with synthetic products the compounds G1 and G2 (see footnote to Table 1) are presumed to be 2-(*N*-aspartic acid)-2-deoxyglucose and 2-asparagino-2-deoxyglucose. Carson (1956) has shown that the reaction of fructose with amines yields 2-(*N*-alkylamino)-2-deoxyglucoses and crystalline 2-deoxy-2-glycino-D-glucose has now been prepared from glycine and fructose (Anet 1957).

The anionic constituents formed during storage (Table 3) reacted with alkaline silver nitrate and appeared to be sugar mono-esters of malic or citric acid. SM1 and SM2 gave malic acid and sucrose when hydrolysed with cold 0·1N sodium hydroxide and the other compounds in fractions 6–48 gave malic acid and glucose, fructose, or sorbitol. The pairs of glucose and fructose esters,

GM1 and GM2, FM1 and FM2, FM3 and FM4, had different $R_{Glucose}$ values on paper chromatograms. It is suggested that the compounds in fractions 1–14 have the structure ROOC.CHOH.CH_2.COOH and those in fractions 17–48 the structure HOOC.CHOH.CH_2.COOR by analogy with β- and α-hydroxybutyric acids whose pK_1 values are 4·29 and 4·12 (Heilbron and Bunbury 1953). The

TABLE 1

ATION-EXCHANGE CHROMATOGRAM FOR FREEZE-DRIED APRICOTS STORED FOR 16 MONTHS AT 25 °C AND 70 PER CENT. R.H.

Fraction No.	Fraction Volume (ml)	Contents of Fractions*†
1–3	2	F1, F1*a*
4–36	,,	F1
37–39	,,	F4, F1, F2, F1*b*, F1*c*, F1*d*
40–66	,,	F4, F3, F1*d*
67	,,	F5, F7, F4, F6, F1*e*, F4*b*, F4*c*
68	,,	F7, F6, F5, F4
69	,,	F7, F6, F8, F9
70	1·7	F7, F8, aspartic acid, F9
71–75	,,	Aspartic acid
76	,,	Aspartic acid, traces of serine, threonine, and proline
77	,,	Aspartic acid, serine, glutamic acid, threonine, proline, traces of asparagine
78–79	,,	Serine, asparagine, proline, traces of glutamic acid and threonine
80–86	,,	Mainly asparagine some proline and serine
87	,,	Asparagine, traces of alanine, serine, proline, and valine
88–89	,,	Alanine, glycine, valine, traces of asparagine and leucine
90–92	2·4	Alanine, glycine, valine, leucine, F11, phenylalanine, a peptide
93	,,	γ-Aminobutyric acid, N1, F4*d*, traces of alanine, leucine, and F11
94	3	N1, N2, (ammonia$_h$), traces of γ-aminobutyric acid and histidine
95	,,	N2, N3, ammonia, traces of γ-aminobutyric acid
96	,,	Ammonia, traces of N3
97	,,	Ammonia‡

* See Table 2 for key to the code names.

† Components listed in order of decreasing concentration. G1, G2, F4*a*, tyrosine, β-alanine, and F10 were not detected in this run, but could be seen when suitable fractions from larger runs were re-run on cation-exchange resins.

‡ Faint traces of arginine and an unknown amino acid emerged after ammonia.

pairs of glucose or fructose esters within these two groups presumably result from the esterification of different hydroxyl groups in the sugar residue. Hydrolytic experiments showed that there was also a group of sugar mono-esters of citric acid in the browned fruit (fractions 57–69, Table 3).

Quantitative determinations were made of the ionic constituents before and after storage and the results obtained are shown in Table 4. The striking

feature of these results is that in all cases the free amino acid or organic acid lost was approximately equal, on a molar basis, to the amino acid-deoxyfructoses (and related compounds) or sugar mono-esters formed. The amino acid results

TABLE 2

CHROMATOGRAPHIC BEHAVIOUR OF SOME PRODUCTS FROM THE REACTION OF AMINO ACIDS WITH GLUCOSE AND FRUCTOSE

Compounds in Order of Displacement on "Dowex 50-X4" Resin*	$R_{Glucose}$†
F1*a* .....By-product from L-aspartic acid and D-glucose	0·12
G1 .....2-(*N*-L-Aspartic acid)-2-deoxy-D-glucose	0·60
F1 .....1-(*N*-L-Aspartic acid)-1-deoxy-D-fructose	0·48
F1*b* .....By-product from L-aspartic acid and D-glucose	0·67
F1c .....By-product from L-aspartic acid and D-glucose	0·81
F2 .....1-Deoxy-1-DL-threonino-D-fructose	0·87
F4*a* .....By-product from L-asparagine and D-glucose	0·22
G2 .....2-L-Asparagino-2-deoxy-D-glucose	0·37
F1*d* .....By-product from L-aspartic acid and D-glucose	1·45
F3 .....1-Deoxy-1-DL-serino-D-fructose	0·50
F4 .....1-L-Asparagino-1-deoxy-D-fructose	0·35
F5 .....1-Deoxy-1-L-prolino-D-fructose	1·00
F1*e* .....By-product from L-aspartic acid and D-glucose	0·17
F4*b* .....By-product from L-asparagine and D-glucose	0·45
F4c .....By-product from L-asparagine and D-glucose	0·60
F6 .....1-Deoxy-1-DL-valino-D-fructose	1·60
F7 .....1-L-Alanino-1-deoxy-D-fructose	0·85
F8 .....1-Deoxy-1-glycino-D-fructose	0·54
F9 .....1-Deoxy-1-L-leucino-D-fructose	2·15
1-Deoxy-1-DL-phenylalanino-D-fructose‡	1·90
1-Deoxy-1-L-trysino-D-fructose‡	1·15
Aspartic acid§	
Leucine§	
F10 .....1-β-Alanino-1-deoxy-D-fructose	1·05
β-Alanine§	
F11 .....1-(*N*-γ-Aminobutyric acid)-1-deoxy-D-fructose	1·30
F4*d* .....By-product from L-asparagine and D-glucose	0·90
γ-Aminobutyric acid§	
N1 .....Derived from ammonia and D-glucose	0·70
N2 .....Derived from ammonia and D-glucose	0·93
N3 .....Derived from ammonia and D-glucose	0·63
Ammonia§	

* Synthetic compounds were derived from L- or DL-amino acids as shown; presumably all the amino acids in the fruit were in the L-form.

† $R_{Glucose}$ values on paper chromatograms run in *n*-butanol–acetic acid–water (4 : 1 : 1 v/v).

‡ Synthetic compounds not so far detected in fruit.

§ Included in table to show order of displacement relative to amino acids and ammonia.

have now been confirmed (Ingles and Reynolds 1957, Part IV of this series, unpublished data) by means of elution chromatography on ion-exchange resins (Moore and Stein 1954*a*). The relative rates of reaction of aspartic acid and

asparagine could not be determined because the amide group of asparagine was partially hydrolysed on the columns, but these two acids together had reacted more rapidly than other amino acids. Proline appeared to be unchanged but the detection of traces of deoxyprolinofructose (Tables 1 and 2) showed that some reaction had occurred.

Sucrose, glucose, and fructose were determined on the original apricot purée and the freeze-dried purée stored for 16 months. After allowing for the sugars combined in esters and amino acid derivatives 11 mmoles of glucose and

TABLE 3

DISPLACEMENT CHROMATOGRAM OF ANIONS FROM FREEZE-DRIED APRICOTS STORED FOR 16 MONTHS AT 25 °C AND 70 PER CENT. R.H.

Fraction No. (each 2·5 ml)	Contents of Fractions*†
1–5	SM1
6–7	SM1, GM1, GM2, FM1, FM2
8–14	GM1, GM2, FM1, FM2
15–16	SM2, GM1, GM2, FM1, FM2
17–31	SM2
32	SM2, GM3, SbM1, FM3, FM4
33–35	GM3, SbM1, FM3, FM4
36–46	Quinic acid, GM3, FM3, FM4, SbM1
47–48	Quinic acid, succinic acid, FM4, FM3, GM3, SbM1
49–56	Formic acid,‡ traces of succinic and sugar mono-esters of citric acid
57–69	Sugar mono-esters of citric acid
70	Malic acid, sugar mono-esters of citric acid
71–167	Malic acid
168	Malic acid, traces of mucic and citric acids
169	Citric acid, traces of mucic acid
170–189	Citric acid
190	Citric acid, phosphoric acid
191–196	Phosphoric acid, traces of unknown
197	Phosphoric acid, traces of unknown, hydrochloric acid
198	Hydrochloric acid (displacing acid)

* SM, GM, FM, and SbM denote sucrose, glucose, fructose, and sorbitol mono-esters of malic acid.

† Components are listed in order of decreasing concentration.

‡ It is presumed that the sodium acetate used to regenerate the columns contained traces of sodium formate.

8 mmoles of fructose per 100 g dry weight of fruit were not accounted for. Total hexoses were determined on equilibrated freeze-dried peach purée before and after storage for 15 months and, after allowing for combined sugars (Table 4), 28 mmoles of hexose were not accounted for. No allowance has been made for the reaction, which presumably occurred, between glucose and any free amino groups in the fruit protein (cf. Lea 1951). It is estimated that the concentration of these groups (as a percentage of the fruit) would be unlikely to exceed one-tenth of the concentration of free amino acids so that no more than 1–2 mmoles of glucose (per 100 g dry weight of fruit) would be involved.

These results make it evident that little, if any, amino acid or organic acid residues had been incorporated in the brown pigments whereas a relatively large quantity of hexoses could have been involved. Weast and Mackinney (1941) isolated the pigment from dried apricots which had become black after

TABLE 4

IONIC CONSTITUENTS IN APRICOT AND PEACH PURÉES BEFORE AND AFTER STORAGE AT 25 °C AND 70 PER CENT. R.H.

Constituents As mmoles per 100 g Dry Wt. of Fruit	Apricots			Peaches	
	Before Storage	Stored 4 Months	Stored 16 Months	Before Storage	Stored 15 Months
Cationic constituents					
Asparagine	7·3	3·0	1·3	10·9	2·8
Aspartic acid	1·8	1·2	1·0	3·0	1·1
Proline	0·5	0·5	0·5	Trace	Trace
Total amino acids	11·4	7·0	3·9	17·1	7·0
F1–F9, F1*a*–F1*e*, F4*a*–F4*c*, G1, G2* (calc. as asparagino-deoxyfructose)	0	4·3	7·1	0	9·8
F10, F11, F4*d*, N1–N3* ..	0	<0·5	<0·5	0	<0·5
Ammonia	4·6	N.D.	4·1	1·1	2·6
Anionic constituents					
Quinic acid	3	N.D.	N.D.	17·9	16·4
Galacturonic acid	0	0	0	1·5	1
Malic acid	50·5	44	42	14·5	11·8
SM1 plus SM2†	0	3·3	3·5	0	1·1
GM1–GM3, FM1–FM4, SbM1†	0	3·3	2·7	0	1·1
Citric acid	9·8	9·0	8·4	9·9	8·3
Sugar mono-esters of citric acid	0	1–2	1–2	0	1–2

* Products from the reaction of glucose and fructose with amino acids and ammonia (Table 2).
† Sucrose, glucose, fructose, and sorbitol mono-esters of malic acid.

some years storage. The nitrogen content of the pigment indicated the combination of 1 mole of amino acid with 3 moles of sugar, a result similar to that obtained for pigments isolated from synthetic amino acid-sugar reactions (Weast and Mackinney 1941 ; cf. also Hodge 1953). However, the more recent work of

Haas and Stadtman (1949) has shown that, in fruit, organic acids are involved in browning reactions as well as amino acids and sugars. It therefore becomes necessary to study the relative rates of reaction of these compounds with each other, and with the primary reaction products now described, in order to determine which reactions produce the brown pigments present at the relatively early, but very important, stage of browning now being investigated.

II. Experimental

Solutions were evaporated under reduced pressure (bath temp. 30–40 °C) using rotary evaporators.

(*a*) *Preparation of Frozen, Freeze-Dried, and Equilibrated Freeze-Dried Fruit Purées.*—Ripe whole apricots (or peaches) were heated in flowing steam for 3–5 (or 8–10) min to inactivate enzymes, pitted, and put through a Fitzpatrick disintegrator ($\frac{1}{8}$ in. mesh screen). The resultant purée (pH 3·5–3·6) was frozen in 1 lb sealed cans and stored at —20 °C. Purée was freeze-dried in a tray-drier as described by Mellor (1954); the final moisture content was 1–4%. Equilibration to 70% R.H. (21% moisture) was accomplished over saturated aqueous strontium chloride at 25 °C under reduced pressure.

(*b*) *Preparation of Fruit Extracts.*—Thawed purée (340 g) or reconstituted, equilibrated, freeze-dried purée (75 g with 280 ml water) was mixed with 95% ethanol (1570 ml) in 3 or 4 batches in a Waring blendor. The filtered 80% ethanolic extract and washings were (i) concentrated to a syrup which was taken up in water and filtered through "Dicalite 4200" (The Dicalite Co. N.Y.), or (ii) concentrated to half volume, or (iii) used untreated.

(*c*) *Separation of Soluble Constituents.*—(i) *Cationic, Anionic, and Neutral Fractions.* The cations in the fruit extracts were collected on "Dowex 50-X4" (100 g column, hydrogen form) and the anions on "Dowex 1-X4" (100 g, acetate form). "Amberlite 1R-4B" (100 g, base form) was used to free the neutral constituents from the acetic acid displaced from the "Dowex 1". These operations were carried out in either water or de-aerated 50 or 80% ethanol, depending on the fruit extract used, and the neutral constituents were washed through with the same solvent.

(ii) *Cationic Constituents.* The "Dowex 50-X4" column was washed thoroughly with water, or with 50 or 80% ethanol followed by water, and then attached to a set of columns (30 to 0·3 g) of the same resin. The cations were displaced with aqueous 0·075N sodium hydroxide. The effluent was collected in fractions which were examined by paper chromatography on washed (Reynolds 1957) No. 1 Whatman paper using phenol–borate buffer pH 9·3 (Levy and Chung 1953) (4 : 1 w/v) and *n*-butanol–acetic acid–water (4 : 1 : 1 v/v) as solvents. The spots were revealed with (1) ninhydrin, (2) silver nitrate–sodium hydroxide (Ash and Reynolds 1954, p. 436), and (3) triphenyltetrazolium chloride–sodium hydroxide (Trevelyan, Proctor, and Harrison 1950) allowed to develop 10 min in the cold.

(iii) *Anionic Constituents.* The "Dowex 1-X4" column was attached without further washing to a set of columns (30 to 0·3 g) of the same resin in the acetate form. The anions were displaced with aqueous 0·1N hydrochloric acid. The effluent was collected in fractions which were examined by paper chromatography using methyl ethyl ketone–cineole–aqueous formic acid (53% w/w) (50 : 50 : 36 v/v) and, for certain fractions, *n*-butanol–acetic acid–water (4 : 1 : 1 v/v) as solvents. The spots were revealed with (1) silver nitrate or silver nitrate–sodium hydroxide (Anet and Reynolds 1955*a*) and (2) aniline phosphate or naphthoresorcinol–trichloroacetic acid (Ash and Reynolds 1954, p. 436).

(iv) *Neutral Constituents.* The de-ionized solutions were concentrated and examined by paper chromatography using *n*-butanol–acetic acid–water (4 : 1 : 1 v/v), ethyl acetate–pyridine–water (8 : 2 : 1 v/v), and ethyl acetate–acetic acid–water (3 : 1 : 3 v/v, organic layer) as solvents. The spots were revealed with silver nitrate–sodium hydroxide, aniline phosphate, and naphthoresorcinol–trichloroacetic acid.

(*d*) *Samples Used.*—(1) Frozen apricot purée, var. Trevatt picked at Wedderburn, N.S.W., December 1952; (2) equilibrated freeze-dried purée prepared from sample 1; (3) sample 2 stored 4 months at 25 °C, light brown in colour; (4) sample 2 stored 13 months at 25 °C, mid-brown in colour; (5) sample 2 stored 16 months at 25 °C, mid-brown in colour; (6) frozen peach purée, var. Blackburn Elbert picked at Wedderburn, January 1951; (7) equilibrated freeze-dried purée prepared from sample 6; (8) sample 7 stored 12 months at 25 °C, dark brown in colour; (9) sample 7 stored 15 months at 25 °C, dark brown in colour.

(*e*) *Soluble Constituents of Frozen Apricot and Peach Purées.*—Samples 1 and 6 contained all the constituents previously found in ethanolic extracts of fresh apricots and peaches (cations, Reynolds 1957; anions, Anet and Reynolds 1955*a*, 1955*b*; neutral constituents, Ash and Reynolds 1955*a*, 1955*b*). No other constituents were detected.

(*f*) *Cationic Constituents of Browned, Equilibrated, Freeze-Dried Purées.*—Samples 3, 5, and 9 were examined and found to have the same qualitative composition. They contained all the amino acids found in samples 1 and 6 and at least 25 additional compounds, as shown in Tables 1 and 2. The mixtures in fractions 37–39, 67–70, and 90–96 were separated further using a larger quantity (260 g) of sample 8; the initial separation was carried out as described and then the relevant fractions were combined and re-run on the same type of column.

F1–F11 had the properties of 1-(*N*-amino acid)-1-deoxy-D-fructoses. On paper they reacted strongly with ninhydrin, silver nitrate–sodium hydroxide, and triphenyltetrazolium chloride. In solution, as collected from the columns, they reduced alkaline methylene blue, 2,6-dichlorophenolindophenol, or *o*-dinitrobenzene at the same rate as 1-deoxy-1-morpholino-D-fructose (Hodge and Rist 1953). When heated for 1 hr at 100 °C in 1N hydrochloric acid (Gottschalk 1952) or 0·1N acetic acid they gave a free amino acid, hydroxymethylfurfural, a residue of the unchanged compound, and a number of unidentified compounds which reacted with aniline phosphate (F1 (from sample 3) contained N, 4·3%. Calc. for $C_{10}H_{17}O_9N$: N, 4·7%. F4 (from sample 8), contaminated with traces of F1*d* and F3, contained N, 8·7%. Calc. for $C_{16}H_{18}O_8N_2$: N, 9·5%). Only F1 was obtained pure by displacement chromatography. The other compounds were separated on paper chromatograms and eluted. The parent amino acid obtained after hydrolysis was identified by paper chromatography (one- and two-dimensional). The R_G value and order of displacement from ion-exchange resins were compared with those of the synthetic compound prepared as described below. The identity of these compounds and their chromatographic behaviour are shown in Table 2.

F1*a*–F1*d* gave the same reactions on paper as F1–F11. F1*e* gave a characteristic green colour with ninhydrin; it reacted strongly with silver nitrate–sodium hydroxide but gave a very weak reaction with triphenyltetrazolium chloride. The R_G values and order of displacement of F1*a*–F1*e* (Table 2) were the same as those of the by-products of the reaction between aspartic acid and glucose (see synthetic experiments below). F1*d* and F1*e* were eluted from paper chromatograms; F1*d* gave aspartic acid when heated with hydrochloric acid, as did F1*e* when heated with acetic acid. F4*a*–F4*d* gave the same reactions on paper as F1–F11 and their chromatographic behaviour (Table 2) was the same as that of the by-products of the asparagine-glucose reaction. F4*d* was eluted from paper chromatograms; when heated with acetic acid it gave first F4 and then asparagine.

G1 and G2 reacted with ninhydrin and alkaline silver nitrate but reacted only weakly with triphenyltetrazolium chloride. Their chromatographic behaviour (Table 2) was the same as that of the 2-deoxy-D-glucose derivatives of aspartic acid and asparagine.

N1–N3 reacted with ninhydrin and silver nitrate–sodium hydroxide, but only N1 reacted s rongly with triphenyltetrazolium chloride. Their chromatographic behaviour (Table 2) was the same as that of the products of the ammonium chloride–glucose reaction.

N3 gave a normal spot but other compounds of comparable basicity (F10, F11, F4*d*, N1, N2) gave characteristic spots with a higher concentration at the leading end and a trail which varied with the concentration and from one chromatogram to another. The R_G values for these compounds were calculated from the zone of highest concentration.

(*g*) *Synthesis of Reaction Products of Glucose and Amino Acids (and Ammonia).*—The only products obtained crystalline were 1-(β-alanino)-, and 1-glycino-1-deoxy-D-fructose which are

described elsewhere (Anet 1957). The code numbers are those used for the cationic constituents of the fruit.

The amino acid (1 mole) and glucose (1–4 moles) were dissolved in water; the solution was concentrated to 80% solids and stored at 50 °C until it became a dark (transparent) brown (3–40 days). The mixture containing γ-aminobutyric acid was adjusted to pH 3·5, L-glutamic acid was used as the mono-sodium salt, and sorbitol (10 moles) was used to bring asparagine into solution. Alternatively (cf. Reynolds 1957, Part III of this series, unpublished data), the amino acid (1 mole), glucose (1–6 moles), sodium metabisulphite (0·5–1·5 moles), and water (10%) were heated at 100 °C for 0·5–3·5 hr. In all cases the reaction mixture was dissolved in 70% ethanol and the basic products were collected on a column of " Dowex 50-X4 " and separated by displacement chromatography.

The main product of the reaction was a 1-(*N*-amino acid)-1-deoxy-D-fructose (F). Three by-products (F*a*–Fc) of unknown constitution with the same reactions as F were always present in small quantity; F*a* had a lower R_G value than F and emerged before it, F*b* and Fc had higher R_G values than F and emerged after it. The chromatographic behaviour of F1*a*–F1c (from L-aspartic acid) and F4*a*–F4c (from L-asparagine) is shown in Table 2. L-Aspartic acid gave two additional by-products, F1*d* and F1e (Table 2); F1*d* reacted like F1 but F1e gave a green colour with ninhydrin and a weak reaction with triphenyltetrazolium chloride. L-Asparagine was partially hydrolysed during the reaction and gave all the products expected from the reaction of glucose with L-aspartic acid, L-asparagine, and ammonium chloride. In addition, L-asparagine gave the relatively strong base, F4*d* (Table 2, hydrolysis above). Ammonium chloride and glucose (approx. pH 4·0) gave a low total yield of N1, N2, and N3 (Table 2). (F1 contained N, 4·7%. Calc. for $C_{10}H_{17}O_9N$: N, 4·7%. F4 contained N, 9·2%. Calc. for $C_{10}H_{18}O_8N_2$: N, 9·5%).

The normal product (R_G 0·75) from glucose and L-glutamic acid was obtained in small yield when displaced rapidly from columns of " Dowex 50-X4 ". It was contaminated by a compound (R_G 1·50) (cf. Borsook, Abrams, and Lowy 1955) which reacted with silver nitrate–sodium hydroxide, but not with ninhydrin and only weakly with triphenyltetrazolium chloride. The effluent from these columns, before any bases emerged, was collected on " Dowex 1-X4 " (acetate form) and displaced. The fractions gave one spot (R_F 0·07) in methyl ethyl ketone–cineole–formic acid–water and two reducing (silver nitrate–sodium hydroxide) spots with a trail between (R_G 1·50–1·84) in butanol–acetic acid–water. The untreated reaction mixture from glucose and mono-sodium glutamate was run on paper chromatograms and the only reducing spots were glucose and the normal reaction product (R_G 0·75).

(*h*) *Reaction Products of Fructose and* L-*Asparagine.*—Fructose (20 g), sodium metabisulphite (8 g), L-asparagine (6 g), and water (3 ml) were heated at 100 °C for 1·5 hr. The reaction mixture was dissolved in 70% ethanol and the basic products were collected on a column of " Dowex 50-X4 " and separated by displacement chromatography. The main products, in the order of their emergence from the columns, were 2-(*N*-L-aspartic acid)-2-deoxy-D-glucose (G1; R_G 0·60), 1-(*N*-L-aspartic acid)-1-deoxy-D-fructose (F1), 2-L-asparagino-2-deoxy-D-glucose (G2; R_G 0·37), 1-L-asparagino-1-deoxy-D-fructose (F4). The total yield of reaction products was *c.* 5%. G1 and G2 reacted with ninhydrin and alkaline silver nitrate and only weakly with triphenyltetrazolium chloride.

(*i*) *Anionic Constituents of Browned, Equilibrated, Freeze-Dried Purées.*—Samples 3, 5, and 9 contained all the organic acids found in samples 1 and 6, together with sugar derivatives as shown in Table 3.

The new acids had the properties of sugar (and sorbitol) mono-esters of malic and citric acids. They gave black spots with silver nitrate–sodium hydroxide and typical ketose or aldohexose reactions with naphthoresorcinol–trichloroacetic acid or aniline phosphate. SM1 and SM2 gave malic acid, glucose, and fructose when heated for 1 hr at 100 °C with 0·1N hydrochloric acid; they gave malic acid and sucrose with 0·1N sodium hydroxide at room temperature. The mixtures GM1, GM2, FM1, FM2 (fraction 10, Table 3), and GM3, SbM1, FM3, FM4 (fraction 34, Table 3) gave malic acid, glucose, and fructose when hydrolysed with either acid or alkali. Fraction 34 also yielded sorbitol on hydrolysis. R_G values for these compounds in *n*-butanol–

acetic acid–water were as follows: SM1, 0·74; SM2, 0·62; GM1, 1·00; GM2, 1·25; GM3, 0·87; SbM1, 1·00; FM1, 1·38; FM2, 1·58; FM3, 1·10; FM4, 1·20.

The compounds in fractions 57–69 (Table 3) were not clearly separated but they gave ketose or aldohexose reactions and yielded citric acid, fructose, and glucose when hydrolysed with 0·1N hydrochloric acid.

(*j*) *Neutral Constituents of Browned Equilibrated, Freeze-Dried Purées.*—All the sugars and polyols present in samples 1 and 6 were detected in samples 3, 5, and 9. The paper chromatograms also showed traces of material reacting with silver nitrate–sodium hydroxide and aniline phosphate. These traces were usually streaks and were not investigated.

(*k*) *Quantitative Determination of Cationic and Anionic Constituents Before and After Browning.*—Ammonia was determined (Varner *et al.* 1953) in 80% ethanolic extracts. Appropriate fractions from the ion-exchange chromatograms (cf. Tables 1 and 3) were combined to determine the following: (1) Total amino acid with ninhydrin (Moore and Stein 1954*b*); (2) aspartic acid by electrometric titration; (3) asparagine as amide (Varner *et al.* 1953); (4) proline with acidified ninhydrin (Chinard 1952); (5) basic reaction products emerging before aspartic acid (F1–F9, F1*a*–F1*e*, F4*a*–F4*c*) by weight calc. as asparagino-deoxyfructose; (6) other basic reaction products (F10, F11, F4*d*, N1–N3) by visual inspection of paper chromatograms; (7) malic and citric acids, and quinic acid in samples 6 and 9, by titration; (8) MS1, MS2, and the mixture MG1, MG2, MF1, MF2, by weight; (9) other sugar mono-esters of malic and citric acids by visual inspection of paper chromatograms.

(*l*) *Quantitative Determination of Sugars Before and After Browning.*—(i) *Frozen Apricot Purée and Freeze-Dried Purée 16 Months* (*Samples 1 and 5*). Total sugars were determined with anthrone (Fairbairn 1953) in the neutral fractions and calc. as glucose; sample 1, 339 and sample 5, 300 mmoles/100 g dry wt. of fruit. The neutral fractions were run on paper chromatograms developed with ethyl acetate–acetic acid–water (3 : 1 : 3 v/v) and the sugars were eluted and determined with anthrone. The results for samples 1 and 5, expressed as mmoles/100 g dry wt. of fruit were: sucrose 100 and 67, glucose 79 and 86, and fructose 60 and 80. The decrease in sucrose (33 mmoles) was assumed to be due to ester formation (4 mmoles, Table 4) and hydrolysis to glucose (29 mmoles) and fructose (29 mmoles). The total glucose available for reaction was then 108 mmoles and the loss 22 mmoles, of which 11 mmoles were combined with amino and organic acids (Table 4). The total fructose available was 89 mmoles and the loss 9 mmoles with approximately 1 mmole present in combination. Xylose was estimated visually on paper chromatograms treated with silver nitrate–sodium hydroxide; sample 1 contained 1 mmole and sample 5 less than 0·5 mmole/100 g dry wt. of fruit.

(ii) *Freeze-Dried Apricot Purée Stored 0 and 13 Months* (*Samples 2 and 4*) *and Freeze-Dried Peach Purée Stored 0 and 15 Months* (*Samples 7 and 9*). Freeze-dried purée was taken from −20 °C and brought to 70% R.H. at 25 °C along with the sample of stored purée. 80% ethanolic extracts were prepared. The extract (6 ml) was diluted to 500 ml with water and run through columns of (1) "Dowex 50-X4" (5 g), (2) "Dowex 1-X4" (3 g) in the acetate form, (3) "Amberlite 1R-4B" (5 g). Total sugars (as glucose) were determined with anthrone in the final effluent: sample 2, 377; sample 4, 349; sample 7, 379; sample 9, 335 mmoles/100 g dry wt. of fruit. Apparent total sugars were determined on the untreated extract and on extracts which had been passed through column (1) and columns (1) and (2). De-ionization did not affect the results obtained for samples 2 and 7 but it reduced the values for samples 4 and 9 by 5–7·5%; adsorption was greatest on the "Dowex 1-X4" column which retained 3·5–6·5% of the apparent sugar.

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CHEMISTRY OF NON-ENZYMIC BROWNING

II. SOME CRYSTALLINE AMINO ACID-DEOXY-SUGARS

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Summary

From the reactions of glycine with glucose, fructose, and xylose, 1-deoxy-1-glycino-D-fructose, 2-deoxy-2-glycino-α-D-glucose, and 1-deoxy-1-glycino-D-*threo*pentulose respectively have been isolated pure and crystalline. Similarly glucose and β-alanine gave 1-β-alanino-1-deoxy-D-fructose.

I. Introduction

A few compounds of the type 1-(*N*-amino acid)-1-deoxy-D-fructose have been observed in hog liver and were found to stimulate incorporation *in vitro* of amino acids into the protein of rabbit reticulocytes (Borsook, Abrams, and Lowy 1955). Anet and Reynolds (1956, 1957) isolated some members of this series from stored dried apricots and peaches. These compounds are proposed intermediates in Hodge's (1953) theory of browning of sugar-amine systems.

The first synthesis and isolation (75–90 per cent. purity) of a 1-(*N*-amino acid)-1-deoxy-D-fructose was achieved by Gottschalk (1952) by refluxing a solution of DL-phenylalanine and glucose in methanol. Abrams, Lowy, and Borsook (1955) synthesized nine members of this series by the same method purifying small quantities by elution chromatography on cation-exchange resins. They obtained them as non-crystalline slightly hygroscopic powders. Some (*N*-amino acid)-deoxy-sugars derived from various amino acids and sugars have now been obtained crystalline.

The method of preparation consisted of heating a reducing sugar and an amino acid as a syrup, and separating the product from the unchanged amino acid and sugar by displacement chromatography on cation-exchange resins (Anet and Reynolds 1957). The addition of sodium bisulphite (Reynolds 1957, Part III of this series unpublished data) to the reaction mixture increased the yield of the product. The protective action of the bisulphite was most noticeable with the more reactive pentose derivative.

The compounds prepared from glucose and glycine, and from glucose and β-alanine, had similar properties (Table 1) to 1-deoxy-1-morpholino-D-fructose prepared according to the method of Hodge and Rist (1953). When heated with acid they decompose slowly rather than hydrolyse, liberating the parent

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TABLE 1

COLOUR REACTIONS OF SOME AMINO-DEOXY-SUGARS

Reagents	Deoxy-glycino-fructose	β-Alanino-deoxy-fructose	Deoxy-morpholino-fructose	Deoxy-glycino-*threo*-pentulose	Deoxy-glycino-glucose	Glucos-amine	Glucose	Fructose
Methylene blue,* time to colourless (min)	3	2	0·5	0·1	100	70	>200	30
2, 6 - Dichlorophenolindophenol,* time to colourless (min)	4	2	2	0·1	140	100	>200	60
O-Dinitrobenzene,† time for violet colour to appear (min)	2	2	3	0·1	100	∞	100	10
Alkaline silver nitrate‡ ..	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong
Alkaline triphenyltetrazolium chloride‡	Strong	Strong	Strong	Strong	None	None	None	Weak
Ninhydrin‡	Strong	Strong	Strong	Strong	Strong	Strong	None	None
Elson-Morgan reagent‡§ ..	Red	Red	Blue	Red	Mauve	Red	None	None
Aniline phosphate‡||	Weak brown	Weak brown	Weak brown	Red brown	Brown	Brown	Brown	Brown

* Methylene blue (0·01%) or 2,6-dichlorophenolindophenol (0·016%), sodium hydroxide (0·016N), and the substance (0·0067M) after mixing (cf. Kuhn and Birkofer 1938).

† *O*-Dinitrobenzene (approx. 1/20 of saturation), sodium hydroxide (0·4N), and the substance (0·008M) after mixing (cf. Fearon and Kawerau 1943).

‡ These tests were done on paper chromatograms.

§ Partridge (1948).

|| Ash and Reynolds (1954).

amino acid, hydroxymethylfurfural, and unidentified products, but no glucose. Thus they are not the glycosyl-(amino acid) but the Amadori rearrangement products, 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose. Whether these compounds and 1-deoxy-1-morpholino-D-fructose exist in the furanose or pyranose form is not known but their optical rotations would indicate that they all have the same form.

The reaction between xylose and glycine gave the lower homologue, 1-deoxy-1-glycino-D-*threo*pentulose, which had stronger reducing properties (Table 1) than the fructose derivatives.

Fructose reacted only slowly with glycine giving one main product, 2-deoxy-2-glycino-α-D-glucose, which had similar properties to those of the 2-alkylamino-2-deoxy-α-D-glucoses prepared by Carson (1955*a*, 1955*b*, 1956) from fructose and alkylamines. Their molecular rotations in water extrapolated to zero time were similar, 2-deoxy-2-glycino-α-D-glucose gave 25,200 and 2-*n*-butylamino-2-deoxy-α-D-glucose, 25,700. The molecular rotations taken at equilibrium were also similar. Glucose derivatives, but no mannose derivatives have been isolated from the reaction of fructose with either ammonia or amines. The high positive rotation (+96°) of 2-deoxy-2-glycino-α-D-glucose also indicates the glucose rather than the mannose configuration, and the negative mutarotation (+96→+75°) suggests an α-configuration. Unlike fructosylalkylamines, these compounds are stable in the dry state and are not easily hydrolysed.

II. Experimental

Melting points are uncorrected. Solutions were evaporated under reduced pressure using rotary evaporators.

(*a*) *Chromatography.*—The reaction mixture was dissolved in de-aerated aqueous ethanol (50–70%) and the solution was passed through a column of sulphonated polystyrene resin "Dowex 50-X4", in the hydrogen form, in aqueous ethanol. The column was washed with aqueous ethanol (approx. 10 column vol.), then with water (1–2 column vol.). A set of two to four smaller columns (Partridge and Brimley 1952) of "Dowex 50-X4" in the hydrogen form in water, was placed under the larger column. The products were displaced through the set with 0·1N ammonia and collected in fractions. Fractions which contained essentially one compound were evaporated to dryness.

Each fraction was examined by descending paper chromatography on "No. 1 Whatman" paper with butanol–acetic acid–water (4 : 1 : 1 v/v) as solvent. Three reagents were used to detect the spots: alkaline silver nitrate, alkaline triphenyltetrazolium chloride (Trevelyan, Proctor, and Harrison 1950) in the cold, and ninhydrin.

(*b*) *1-Deoxy-1-glycino-D-fructose.*—D-Glucose (72 g) and glycine (30 g) were dissolved in water and concentrated to a water content of 20–30 g. The mixture was kept at 50 °C for 3 days. The dark brown products were dissolved in ethanol (50%; 2 l.), and the solution was passed through a column of "Dowex 50-X4" (300 g). Ion-exchange chromatography yielded a dark brown crystalline mass (12 g). Three recrystallizations from water gave the 1-glycino-1-deoxy-D-fructose as colourless, non-hygroscopic, large prisms, m.p. 145–146 °C (decomp.), $[\alpha]_D^{20}$ —68·8° (constant 0·5 hr; *c*, 1·2 in water) (cf. $[\alpha]_D^{25}$ —65° for the amorphous product given by Abrams, Lowy, and Borsook 1955) (Found: C, 41·0, 40·9; H, 6·4, 6·4; N, 5·6%. Calc. for $C_8H_{15}NO_7$: C, 40·6; H, 6·4; N, 5·9%). On paper chromatograms the compound showed as one spot $R_{Glucose}$ (R_G) 0·54. Colour reactions are shown in Table 1.

§ Partridge (1948).
|| Ash and Reynolds (1954).

(c) *1-Deoxy-1-glycino*-D-threo*pentulose*.—(i) D-Xylose (10 g), glycine (4 g), sodium metabisulphite (3 g), and water (5 ml) were quickly dissolved at 100 °C and evaporated to a thick syrup (90% solids) on a water-bath at 65 °C. The syrup, which was pale yellow, was heated at 100 °C until brown (5 min). The brown syrup was dissolved in ethanol (70%; 570 ml) and the solution (light brown) was passed through a column of " Dowex 50-X4 ". Ion-exchange and paper chromatography showed only one main product, R_G 0·78. Recrystallization from water yielded 1 g of colourless prisms, m.p. 135 °C (decomp.), $[\alpha]_D^{20}$ −10° (no mutarotation; *c*, 0·4 in water) (Found: C, 40·4, 40·5; H, 6·3, 6·2; N, 6·7, 6·7%. Calc. for $C_7H_{13}NO_6$: C, 40·6; H, 6·3; N, 6·8%). Colour reactions of *1-deoxy-1-glycino*-D-threo*pentulose* are shown in Table 1.

(ii) D-Xylose (20 g), glycine (10 g), and water (6 ml) were stirred at 50 °C for 23 hr. Extraction of the products with 70% ethanol left much black tar. The products in the extract (dark brown) were taken up by a column of " Dowex 50-X4 " (30 g). Ion-exchange and paper chromatography showed two sharply separated products present in approximately equal quantity. The first compound to emerge from the columns could not be crystallized and did not react with alkaline triphenyltetrazolium chloride or ninhydrin on paper chromatograms (R_G 0·42). The second compound was identical to the product obtained when sodium metabisulphite was added, m.p. 135 °C (decomp.), $[\alpha]_D^{20}$ −12° (micro) (Found: C, 40·2; H, 6·8%. Calc. for $C_7H_{13}NO_6$: C, 40·6; H, 6·3%).

(d) *2-Deoxy-2-glycino*-α-D-*glucose*.—D-Fructose (72 g) and glycine (30 g) were dissolved in water and concentrated to a water content of 30 g. The mixture was stored at 50 °C for 38 days. The dark brown products were dissolved in ethanol (50%; 2 l.), and the solution was passed through a column of " Dowex 50-X4 " (300 g). Ion-exchange chromatography yielded a dark brown crystalline mass (15 g). Recrystallization from water and rechromatography on ion-exchange columns removed all the colour, yielding the hydrate as colourless needles, m.p. 138–139 °C (decomp.), $[\alpha]_D^{20}$ +96° (0)→+75° (const. 2 hr; *c*, 1·2 in water) (Found: C, 38·0, 37·9; H, 6·8, 6·7; N, 5·3%. Calc. for $C_8H_{15}NO_7.H_2O$: C, 37·6; H, 6·7; N, 5·5%). Drying at 70 °C for 1 hr gave the anhydrous compound (Found: C, 40·4; H, 6·4; N, 5·6%. Calc. for $C_8H_{15}NO_7$: C, 40·5; H, 6·4; N, 5·9%). *2-Deoxy-2-glycino*-α-D-*glucose* was treated with ninhydrin (Stoffyn and Jeanloz 1954) and gave arabinose, in the same way as 2-amino-2-deoxy-α-D-glucose (glucosamine) but unlike *N*-substituted-1-amino-1-deoxyfructoses. On paper chromatograms the compound showed as one spot R_G 0·58. Colour reactions are shown in Table 1.

(e) *1-*β*-Alanino-1-deoxy*-D-*fructose*.—D-Glucose (10 g), β-alanine (4 g), sodium metabisulphite (3 g), and water (1·7 ml) were heated at 100 °C for 0·5 hr. The reaction mixture was dissolved in ethanol (70%; 500 ml) and the solution was passed through a column of " Dowex 50-X4 ". Ion-exchange chromatography yielded one main product which was recrystallized from water as colourless needles, m.p. 153 °C (decomp.), $[\alpha]_D^{20}$ −56·9° (no mutarotation; *c*, 0·5 in water) (Found: C, 42·7; H, 6·8; N, 5·5, 5·4%. Calc. for $C_9H_{17}NO_7$: C, 43·1; H, 6·8; N, 5·8%). On paper chromatograms the compound showed as one spot R_G 1·05. Colour reactions of *1-*β*-alanino-1-dexoy*-D-*fructose* are shown in Table 1.

(f) *Behaviour on Buffered Ion-Exchange Columns*.—All four compounds were eluted from ion-exchange columns (Moore and Stein 1954) as sharp single peaks. The three glycino-compounds emerged in the order -deoxyglucose, -deoxyfructose, and -deoxy*threo*pentulose before aspartic acid. The β-alanino-deoxyfructose emerged with *iso*leucine.

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WATER-SOLUBLE CONSTITUENTS OF FRUIT

VI. THE AMINO ACIDS IN APRICOTS AND PEACHES*

By T. M. Reynolds

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Summary

The amino acids in apricots and peaches were fractionated by displacement chromatography on columns of cation-exchange resins and then examined by paper chromatography. The same 18 amino acids were identified in both species. Except for a much lower concentration of proline in peaches the amino acids were present in similar proportions in the two species.

I. Introduction

The amino acids in apricots have been studied by Joslyn and Stepka (1949) by paper chromatography of an 80 per cent. ethanolic extract of the fruit. These workers detected 10 amino acids (aspartic and glutamic acids, serine, proline, asparagine, glutamine, alanine, valine, leucine (or *iso*leucine), and γ-aminobutyric acid) in Blenheim and Moorpark apricots. The present paper is believed to be the first report on the identity of the amino acids in peaches.

The cationic constituents in an 80 per cent. ethanolic extract of the fruit were collected on a column of cation-exchange resin and fractionated by displacement chromatography on columns of the same resin (Partridge and Brimley 1952). The amino acids in the fractions collected were identified by their order of displacement and their rate of movement and chemical reactions on paper chromatograms.

An ion-exchange chromatogram for apricots is shown in Table 1. Eighteen amino acids were identified, three were tentatively identified (U2, U3, U5, Table 2), and three were not identified (U1, U4, U6, Table 2). Many of these, including U1–U6 and the eight amino acids not detected by Joslyn and Stepka (1949), were present in very low concentrations. Recent work by Ingles and Reynolds (1957, unpublished data) has shown that only eight amino acids (aspartic and glutamic acids, serine, asparagine, proline, alanine, valine, and γ-amibobutyric acid) occurred in quantities exceeding 0·10 mmoles/100 g dry weight of fruit in a sample of apricots which gave an ion-exchange chromatogram (Anet and Reynolds 1957) very similar to that shown in Table 1.

Both varieties of peaches contained the 18 amino acids identified in apricots. The ion-exchange chromatogram shown (Table 1) is typical for both species

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except that the peaches contained much less proline. U2 and U5 were detected in Blackburn Elberta peaches and U3, U4, U6, and another unidentified amino acid (U7, Table 2) were detected in Halehaven peaches. Compounds corresponding with P1–P3 (Table 1) were also detected in peaches and shown to be peptides.

TABLE 1

ION-EXCHANGE CHROMATOGRAM OF AMINO ACIDS IN TREVATT APRICOTS

Fraction No. (each 5 ml)	Contents of Fraction*
1–4	Aspartic acid, traces of U1 and other unknowns
5	Aspartic acid, traces of threonine
6	Aspartic acid, threonine, glutamic acid, serine, traces of U2
7	Threonine, glutamic acid, serine, aspartic acid, traces of U2
8–10	Glutamic acid, serine, threonine, aspartic acid, traces of U2
11	Glutamic acid, serine, traces of aspartic acid, asparagine, proline, and threonine
12–20	Asparagine, proline, decreasing quantities of glutamic acid and serine, traces of aspartic acid†
21–34	Asparagine, proline, traces of serine to fraction 24, traces of aspartic acid to fraction 27
35	Asparagine, proline, traces of glutamine, alanine, and U3
36	Alanine, asparagine, proline, U3, traces of glutamine, glycine, U4, and P1
40	Alanine, valine, glycine, U5, traces of leucine (or *iso*leucine)
41	Valine, alanine, leucine (or *iso*leucine), phenylalanine, U5, tyrosine, P2, cystine, and U6
42	γ-Aminobutyric acid, leucine (or *iso*leucine), phenylalanine, tyrosine, cystine, traces of β-alanine, valine, and P2
43–44	γ-Aminobutyric acid, (ammonia ?), traces of histidine, P3, tyrosine, β-alanine, and unknowns
45–64	Ammonia
65	Ammonia, sodium hydroxide (displacing base), traces of arginine, and unknowns

* Contents listed in decreasing order of concentration. The properties of U1–U6 are shown in Table 2. U7 (Table 2), if present, was not distinguished from valine in apricots, but it emerged just after valine in runs on Halehaven peaches. P1–P3 were shown to be peptides.

† Hydrolysis of asparagine on the columns presumably caused the trail of aspartic acid seen in fractions 12–27.

II. EXPERIMENTAL

(*a*) *Ion-Exchange Chromatography of Amino Acids.*—(i) An aqueous solution was prepared from an 80% ethanolic extract of the fruit (Anet and Reynolds 1955, Section IV (*c*)). The solution from *c.* 1 kg fruit was run through coupled columns of "Zeo Karb 225-4·5 NC" (60 and 30 g, H form) which were then washed with water and attached to a set of columns (10, 3, and 1 g) of the same resin. The amino acids were displaced with 0·075N sodium hydroxide (Partridge and Brimley 1952) and collected in fractions which were examined by paper chromatography. During the displacement dark bands formed on the columns behind the amino acids indicating that some alkali-sensitive compounds were adsorbed on the resin; the fractions containing γ-aminobutyric acid were also discoloured.

(ii) The 80% ethanolic extract prepared from *c.* 1 kg fruit was run through a column of "Zeo Karb 225-10 NC" (170 g, 20–40 mesh, H form, equilibrated in de-aerated 80% ethanol). The column was washed with de-aerated 80% and 50% ethanol and then with water. The amino acids were displaced with 0·75N (Shewan *et al.* 1952), or 0·075N, sodium hydroxide onto a column of "Dowex 50-X4" (30 g) which was then attached to a set of columns (10 g down to 0·3 g) of the same resin. The amino acids were displaced with 0·075N sodium hydroxide as before. There was no discolouration on the columns and all the fractions were colourless.

TABLE 2

PROPERTIES OF SOME AMINO ACIDS DETECTED IN APRICOTS AND PEACHES

Compound*	R_F Values on Paper Chromatograms in Solvents *A* and *B*†‡	Colour Reactions with (1) Ninhydrin (2) Isatin	Comments or Results of other Tests
U1	0·12, 0·32 (Serine —, 0·34)	(1) Purple (2) Pink	——
U2	0·22, 0·83 (Threonine 0·21, 0·54)	(1) Yellow to reddish (2) Bright blue	Presumed to be the methylhydroxyproline isolated (Urbach 1955) from apples
U3	0·44, 0·94 (Alanine 0·25, 0·64)	(1) Yellow to reddish (2) Bright blue	Presumed to be the methylproline isolated (Hulme and Arthington 1952; Hulme 1954) from apples
U4	0·21, 0·69 (Alanine 0·25, 0·64)	(1) Purple	Not readily hydrolysed
U5	0·39, 0·92 (Phenylalanine 0·48, 0·90)	(1) Bright bluish purple, magenta in ultraviolet (2) Bluish green	Presumed to be pipecolinic acid (cf. Morrison 1953; Grobelaar, Zacharius, and Steward 1954)
U6	0·13, 0·61 (Alanine 0·25, 0·56)	(1) Purple (2) Pink	Not methionine sulphone; not a peptide
U7	0·40, 0·77 (Valine 0·44, 0·77)	(1) Purple	An α-amino acid; not methionine; not a peptide

* See Table 1 for order of emergence from cation-exchange resin columns.

† Solvent *A* was *n*-butanol–acetic acid–water (4 : 1 : 1 v/v); solvent *B* was phenol–water (4 : 1 w/v)–ammonia or phenol–borate buffer pH 9·3 (4 : 1 w/v).

‡ R_F values of a known amino acid on the same chromatogram (usually two-dimensional) are shown in parentheses.

(iii) The order of displacement of authentic amino acids from sets of columns of "Dowex 50-X4" was found to be similar to the sequence determined by Partridge and Brimley (1952) for a similar resin. The order of emergence from the columns of some amino acids (asparagine, glutamine, tyrosine, γ-aminobutyric acid) not listed by these authors was as follows: asparagine emerged with proline, glutamine between asparagine and alanine, tyrosine with phenylalanine, and γ-aminobutyric acid between β-alanine and histidine. When the displacement was carried out in 50% ethanol proline and asparagine were well separated, proline emerging first.

(*b*) *Paper Chromatography of Amino Acids.*—Chromatograms were run by the descending method on No. 1 Whatman paper which had been washed with disodium ethylenediaminetetra-acetate solution (0·2%) and water. The solvents used were (i) *n*-butanol–acetic acid–water (4 : 1 : 1 v/v) and (ii) phenol–water (4 : 1 w/v)–ammonia or phenol–borate buffer pH 9·3 (Levy and Chung 1953) (4 : 1 w/v). Each fraction from the columns was examined in both solvents, authentic amino acids being run on all papers. Some fractions were also run on two-dimensional chromatograms developed with the same solvents.

All amino acid spots were revealed with ninhydrin (0·1–0·2% in 95% ethanol as a spray or 0·1% in acetone as a dip). Glacial acetic acid (2–5%, 10% for buffered papers) was added to the reagent immediately before use. The papers were heated for 20 min at 80 °C. Similar results were obtained with spraying and dipping but the latter was found to be more convenient, particularly with high (10%) concentration of acetic acid. The identity of the following amino acids was confirmed on duplicate chromatograms by means of specific reagents: proline with isatin (Acher, Fromageot, and Jutisz 1950); histidine and tyrosine with the Pauly reagent (Ames and Mitchell 1952); arginine with the Sakaguchi reagent (Acher and Crocker 1952); peptides with chlorine–starch–iodide (Rydon and Smith 1952), also sensitive for β-alanine and γ-aminobutyric acid; β- and γ-amino acids were distinguished by the copper test (Crumpler and Dent 1949); oxidation with hydrogen peroxide (Dent 1948) was used as a test for cystine and methionine.

(*c*) *Amino Acids in Apricots.*—Ripe Trevatt apricots from Wedderburn, N.S.W., were examined. An ion-exchange chromatogram of the amino acids and other bases is given in Table 1. The R_F values and colour reactions of the compounds U1–U6 are shown in Table 2. The additional data for U5 and U6 were obtained on fractions from the Halehaven peaches. The compounds P1–P3 are presumed to be the peptides found in Halehaven peaches. Fractions 45–64 gave no spots with ninhydrin or iodine (Brante 1948) but gave a positive test with Nessler's reagent. The presence of ammonium salts in the 80% ethanolic extract of the fruit was confirmed by diffusion tests, which also showed the absence of other volatile bases.

(*d*) *Amino Acids in Peaches.*—Ripe Blackburn Elberta and Halehaven peaches from Wedderburn, N.S.W., were examined. All the amino acids identified in apricots were also identified in both varieties of peaches. The peaches contained much less proline than the apricots but the relative proportions of other amino acids were similar in the two species. U1, U2, and U5 were not detected in Halehaven peaches, but U3, U4, and U6 were present, and also an unknown amino acid, U7 (Table 2). The results of tests on U4, U6, and U7 are shown in Table 2. Only U2 and U5 have so far been detected in Blackburn Elberta peaches.

Fractions from the Halehaven peaches containing the compounds P1–P3 were hydrolysed with 6N hydrochloric acid. P2 and P3 gave aspartic acid, P1 was hydrolysed but the products were not identified. The R_F values of P1–P3 were 0·25, 0·13, and 0·08 in *n*-butanol–acetic acid–water and 0·88, 0·34, and 0·35 in buffered phenol. P3 gave a brown colour, like asparagine, with ninhydrin, while P1 and P2 gave purple coloured spots. P1–P3 were present in ion-exchange chromatograms when the amino acids were collected on "Zeo Karb 225-10 NC" (20–40 mesh).

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SHORT COMMUNICATIONS

DIFFUSION OF THALLIUM (ThC″) IONS IN AIR*

By D. L. Baulch,† J. F. Duncan,† and J. P. Ryan‡

An α-recoil atom has a range in air at atmospheric pressure of 0·1–0·2 mm. Having reached the end of its recoil path, the atom diffuses through the air, and may be collected on a metal plate at a distance several times larger than the range. From the variation of yield with distance between the source and collector one may calculate the diffusion coefficient D. This has been done by Chamié (1934*a*, 1934*b*) and Chamié and Tsien San-Tsiang (1941), who obtained values of D which were very much smaller than predicted from the kinetic theory of gases. Theoretical and practical objections to the range measurements of Chamié (Baulch and Duncan 1957) might have been a contributory factor to this anomaly. We have therefore redetermined the diffusion coefficient of ThC″ by measuring the yield of recoil atoms obtained at different gas pressures on a collector at a fixed distance from a source plate containing an equilibrium mixture of ThB, ThC, ThC′, and ThC″.

The apparatus and procedure were identical with those used in determining the recoil range (Baulch and Duncan 1957), except there was no potential applied to the plates. An exposure time of 20 min was used in all measurements, with gas pressures from about 50 to 200 mm. Counting rates from 2 to 200 counts/min were obtained. Each experimental point (see Fig. 1) is the mean of six to nine determinations. The accuracy varied from 20 per cent. at high recoil yields to 50 per cent. at low recoil yields. The following procedure was used to calculate the diffusion coefficient, using results obtained at pressures above that necessary to reduce the recoil range to at least half the plate separation (20 mm for air and 30 mm for argon).

From Fick's law it may be shown that (see Appendix I)

$$N_h = N_0 \exp\left[-(\lambda/D)^{\frac{1}{2}}h\right], \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

where N_h is the number of atoms reaching the collector at a distance h from the source; N_0 is the number of ThC″ atoms at $h=0$; λ is the radioactive decay constant of ThC″; and D the diffusion coefficient. Since D is inversely proportional to the gas pressure, then

$$N_h = N_0 \exp\left[-(\lambda p/760.D_{760})h\right], \quad \ldots\ldots\ldots\ldots \quad (2)$$

where p is in millimetres. In Figure 1, the gradient according to equation (2) should be $-(\lambda/D_{760})^{\frac{1}{2}}/2{\cdot}303$. The values of D_{760} obtained are compared in

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Table 1 with those quoted by Chamié and Tsien San-Tsiang (1941). On account of the low counting rates used in our work, the values we obtain cannot be regarded as indicating more than an order of magnitude. Agreement with the more accurate work of Chamié is satisfactory.

The diffusion coefficient of thallium ions cannot be theoretically calculated exactly, due to lack of data for the collision diameter. However, if the thallium

Fig. 1.—Plot of log $(10^4.N_h/N_0)$ *against* $h(p/760)^{\frac{1}{2}}$. *A*, air; *B*, argon.

ions were monatomic, ThC″ would have a diffusion coefficient closely similar to that of mercury, for which the calculation may be made exactly (Hirschfelder, Curtiss, and Bird 1954). The calculated values are, for air, $D=0{\cdot}12$ cm²/sec and, for argon, $D=0{\cdot}10$ cm²/sec. It is clear, therefore, that the values obtained experimentally are low by a factor of 1000.

TABLE 1

DIFFUSION COEFFICIENT OF ThC″ RECOIL ATOMS $\times 10^4$ CM²/SEC

Gas	Present Work (at 760 mm)	Chamié and Tsien San-Tsiang (1941) (in air)
Air	1·3	4
Argon	1·8	—

The explanation of this anomaly is not known. But two possibilities may be eliminated at once. Firstly, a decrease by a factor of 1000 requires an increase in the value of the collision cross section by a factor of 33. Thus an aggregate with a diameter of at least 100 Å, containing over 10,000 atoms, would be required. Clusters of 5–10 atoms around alkali-metal atoms are possible (Massey 1952), but such large aggregates would be required to account for the results that Chamié's suggestion of ion aggregates arising from the

presence of an highly ionized medium seems most unlikely. Secondly, the possibility of large aggregates arising from the removal of some of the source material may be rejected for two reasons: (i) an aggregate of the size required would not give a recoil range so close to that expected theoretically, or of the same order of magnitude as that obtained with gaseous sources, such as RaA recoiling from radon (Baulch and Duncan 1957); (ii) no contamination from the ThB parent activity was ever observed in recoil or diffusion experiments.

The anomaly observed by Chamié is therefore confirmed.

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Appendix I

Equation (**1**) has been derived by Rutherford (1905) on the assumption that equilibrium conditions are established between the rate of decay of activity and the rate of arrival of active atoms at a particular plane parallel to the source plate. A more general solution considering non-equilibrium conditions is given.

Consider recoil atoms diffusing from a plane to a collector plate at a distance h from the plane. Let c be the concentration after time t, at any point distance x from the plane. Then from Fick's law we have

$$\frac{\partial c}{\partial t}=D\frac{\partial^2 c}{\partial x^2}-\lambda c,$$

subject to the conditions $c=0$ at $t=0$ $(x>0)$, and $c=c_0$ at $x=0$ $(t>0)$. The number of recoil atoms N collected on the plate is

$$N=\int_h^\infty c(x,t)\mathrm{d}x.$$

Solving for c by use of Laplace transforms, or otherwise,

$$c=\tfrac{1}{2}c_0\,[\exp(-\alpha x)\,\mathrm{erfc}\,(\beta x-\gamma)+\exp(\alpha x)\,\mathrm{erfc}\,(\beta x+\gamma)],$$

where

$$\alpha=(\lambda/D)^{\frac{1}{2}}, \quad \beta=(4Dt)^{-\frac{1}{2}}, \quad \text{and} \quad \gamma=(\lambda t)^{\frac{1}{2}}.$$

Let

$$N=\int_h^\infty c(x, t)\mathrm{d}x=N_1+N_2,$$

where

$$N_1(\alpha, \beta, \gamma)=\tfrac{1}{2}\int_h^\infty \exp(-\alpha x)\operatorname{erfc}(\beta x-\gamma)\mathrm{d}x,$$

$$N_2(\alpha, \beta, \gamma)=N_1(-\alpha, \beta, -\gamma).$$

Using integration by parts and some reduction,

$$N_1=\tfrac{1}{2}\frac{c_0}{\alpha}[\exp(-\alpha h)\operatorname{erfc}(\beta h-\gamma)+\exp(-\gamma^2)\operatorname{erfc}(\beta h)].$$

Hence

$$\begin{aligned}N&=\tfrac{1}{2}\frac{c_0}{\alpha}[\exp(-\alpha h)\operatorname{erfc}(\beta h-\gamma)-\exp(\alpha h)\operatorname{erfc}(\beta h+\gamma)]\\&=\tfrac{1}{2}c_0(D/\lambda)^{\frac{1}{2}}\{\exp[-h(\lambda/D)^{\frac{1}{2}}]\operatorname{erfc}[\tfrac{1}{2}h(Dt)^{-\frac{1}{2}}-(\lambda t)^{\frac{1}{2}}]\\&\quad-\exp[h(\lambda/D)^{\frac{1}{2}}]\operatorname{erfc}[\tfrac{1}{2}h(Dt)^{-\frac{1}{2}}+(\lambda t)^{\frac{1}{2}}]\}.\end{aligned}$$

As $t\to\infty$, using $\operatorname{erfc}(\infty)=0$ and $\operatorname{erfc}(-\infty)=2$,

$$N\to\tfrac{1}{2}c_0(D/\lambda)^{\frac{1}{2}}.\,2\exp[-h(\lambda/D)^{\frac{1}{2}}].$$

The equilibrium value of N is thus

$$N_h=c_0(D/\lambda)^{\frac{1}{2}}\exp[-h(\lambda/D)^{\frac{1}{2}}].$$

Writing N_0 for the value of N_h when $h=0$, we have

$$N_h=N_0\exp[-h(\lambda/D)^{\frac{1}{2}}],$$

which is equation (**1**) referred to above.

The equilibrium activity is

$$\lambda N_h=c_0(D\lambda)^{\frac{1}{2}}\exp[-h(\lambda/D)^{\frac{1}{2}}].$$

This analysis confirms that

$$\lim_{t\to\infty}\int_h^\infty c(x, t)\mathrm{d}x=\int_h^\infty c(x, \infty)\mathrm{d}x,$$

from which the above expression for N_h could have been found immediately.

SURFACE CONDUCTANCE AND THIXOTROPY*

By N. STREET†

It has been shown by Street (1956) that the surface conductance of a suspended material is given by

$$\lambda_s = (F'K - K_1)\frac{\varphi}{S_w},$$

where λ_s, surface conductance (ohm^{-1}),
F', $[(x+1)-\varphi]/x\varphi$,‡
K_1, conductivity of filtrate (ohm^{-1} cm^{-1}),
K, conductivity of suspension (ohm^{-1} cm^{-1}),
φ, fluid fraction of suspension,
S_w, surface area of particles/cm^3 of suspension.

Since this measured surface conductance is equal to that calculated from the measured ζ-potentials (Street 1956), it should be possible to use measurements of surface conductance to calculate the ζ-potential of more concentrated suspensions than is possible by the microelectrophoretic method.

Application

The application of this method to some existing results indicates the desirability of considering the existence of surface conductance phenomena in the interpretation of suspension conductivities.

Schofield and Dakshinamurti (1948) give results for the relative conductivities of bentonite suspensions in potassium bromide solutions of different concentrations, and find that, at concentrations less than N/40, there is an increase in conductivity of about 3 per cent. on addition of the bentonite. However, if the concentration of potassium bromide is greater than N/40 the addition of bentonite to the solution causes a decrease in conductivity. It is also noted that the bentonite is deflocculated in solutions *less* concentrated than N/40 whilst a thixotropic gel is formed in N/10 potassium bromide solution.

These facts were interpreted on the basis of the decrease in conductivity being caused by obstruction by a thixotropic gel network.

The results can be used to calculate the surface conductance of the bentonite in suspension if K_1 is calculated assuming normal conductivity for the electrolyte concentrations given by Schofield and Dakshinamurti (loc. cit.). Since the

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‡ x is a function of the axial ratio of the suspended particles (Fricke 1924).

surface area of the bentonite used was 800 $m^2 g^{-1}$, and the platelets were 10 Å thick, thus the axial ratio is 50 : 1, and x can be calculated (Fricke 1924) to be 0·0446.

Table 1 shows the calculated surface conductances and the ζ-potentials necessary to give them.

These values indicate that the ζ-potential is constant with increasing concentration of electrolyte up to N/40 at which point it drops sharply.

TABLE 1

SURFACE CONDUCTANCE CALCULATED BY ζ-POTENTIALS

KBr (N)	λ_s (10^{-9}/ohm^{-1})	ζ_1* (mV)	ζ_2† (mV)
0·1	Negative	—	—
0·05	1·33	48	58
0·0333	2·61	74	87
0·025	4·82	99	114
0·0167	3·93	98	113
0·0125	4·20	102	117
0·0083	3·55	108	123

* ζ_1, the ζ-potential calculated assuming there is an electro-osmotic contribution to surface conductance.

† ζ_2, the ζ-potential calculated assuming there is no electro-osmotic contribution to surface conductance.

Thus on the basis of this concept the suspension behaviour may be quite normal; as electrolyte concentration increases, the ζ-potential decreases, and this decrease (in ζ-potential) causes a decrease in surface conductance which is reflected in a decrease in suspension conductance. Thixotropic gelation would be expected to occur at some low value of the ζ-potential. The suspension in N/10 potassium bromide solution should have a low ζ-potential and as a consequence a low surface conductance; that it is in fact negative in these results indicates that perhaps gel obstruction is partly responsible for the lower measured suspension conductance at this concentration.

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SOME NON-ALKALOIDAL CONSTITUENTS OF THE BARK OF *TECLEA GRANDIFOLIA* ENGL.*

By E. GELLERT†

As no further work is intended the result of a preliminary examination of the bark of *Teclea grandifolia* Engl., Rutaceae, is reported. *T. grandifolia* is stated (Colonial Products Research Council, London, List of Indigenous Plants of the Colonial Empire of Possible Scientific and Technical Interest, 1945) to be used medicinally in Africa. Related genera, e.g. *Toddalia*, *Skimmia*, and *Casimiroa* have been shown to contain alkaloids. The present examination showed the presence of *c.* 1·2 per cent. alkaloids in *T. grandifolia*. The material, however, was amorphous in character and was not further examined. From the non-basic fractions were isolated the alcohol, tetracosan-1-ol, the triterpenoid, lupeol, a phytosterol which is probably β-sitosterol, and a substance apparently not previously described, $C_{29}H_{46}O$ or $C_{30}H_{48}O$. There was also present an unidentified green syrup with a blue fluorescence.

Experimental

All melting points are corrected. Microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory under the direction of Dr. K. W. Zimmerman.

(i) Milled bark (1·9 kg) of *T. grandifolia* was mixed with lime (0·7 kg) and extracted exhaustively in a Soxhlet with methylene chloride (*A*), then with methanol (*B*). Both extracts were separately evaporated and the residues distributed between aqueous acetic acid and ether.

(ii) From the aqueous acid solutions were recovered after basification 22 g crude base from *A* and 1·5 g from *B*. The products were amorphous and neither the bases nor their salts could be obtained crystalline before or after chromatographing on alumina.

(iii) The non-basic ether-soluble fraction from *A* dissolved in methanol deposited a large quantity of gum, which was discarded. The methanol was removed, and the residue, dissolved in light petroleum, chromatographed on alumina. The column was eluted successively with light petroleum, benzene, and methylene chloride.

(iv) The first light petroleum fractions gave after purification *tetracosan-1-ol*, m.p. 76·5–77 °C (cf. Shah, Phalnikar, and Bhide 1947) (Found : C, 81·1 ; H, 14·2%. Calc. for $C_{24}H_{50}O$: C, 81·3 ; H, 14·2%). The substance gave no depression in m.p. when mixed with an authentic sample prepared by $LiAlH_4$ reduction of tetracosanoic acid.‡ The X-ray diagrams of the two specimens were identical.

(v) The later light petroleum eluates yielded after crystallization from light petroleum a *substance*, m.p. 170·5–171 °C (Found : C, 85·1 ; H, 11·5 ; O, 4·1 ; active " H ", nil %. Calc. for $C_{29}H_{46}O$: C, 84·8 ; H, 11·3 ; O, 3·9% ; for $C_{30}H_{48}O$: C, 84·9 ; H, 11·3 ; O, 3·8%). The substance gives a pink colour changing to pale blue in the Liebermann-Burckhardt test. A

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mixture with stigmasterol ($C_{29}H_{48}O$, m.p. 170–171 °C, cf. Fieser and Fieser 1949) melted at 140 °C.*

(vi) The first benzene eluates gave *lupeol*, m.p. 214–216 °C (Found: C, 84·1; H, 12·1; active "H", 0·23%. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8; active "H", 0·23%). *Acetate*, m.p. 215·5–216·5 °C (Found: C, 82·0; H, 11·2; O, 7·0%. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2; O, 6·8%). *Benzoate*, m.p. 269–270 °C (Found: C, 83·8; H, 10·3; O, 6·0%. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·3; O, 6·0%). The m.p.'s of the lupeol and its derivatives were not depressed when mixed with authentic specimens.

(vii) The later benzene eluates contained a *sterol*, probably β-sitosterol, m.p. 133–134 °C (Found: C, 81·9; H, 12·0; active "H", 0·23%. Calc. for $C_{29}H_{50}O$. $0·5H_2O$: C, 82·2; H, 12·1; active "H", 0·24%). This substance melts at 134–136 °C when mixed with a sitosterol of m.p. 136–137 °C isolated from *Atalaya hemiglauca* (F. Muell.) Benth. (unpublished data). Both give the same colour reaction in the Liebermann-Burckhardt test.

(viii) The methylene chloride eluates yielded a green syrup with a blue fluorescence which would not crystallize.

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